STUDIES ON GUM EXUDATES WITH PARTICULAR REFERENCE TO GUM ARABIC (*ACACIA SENEGAL*) AND OTHER *ACACIA* SPECIES

by

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of

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TO MY FAMILY AND FRIENDS

DECLARATION

I hereby declare that this thesis was composed by myself and that it is based upon the results of original research experiments carried out by me (unless indicated otherwise) within the Chemistry Department, University of Edinburgh, from October 1983 to 30 September 1986. None of the work included in this thesis has been submitted for any other degree or professional qualification.

Some of the analytical data reported in Chapters III, IV and V have been published or are in press; e.g. (a) D.M.W. Anderson, M.C.L. Gill, A.M. Jeffrey and F.J. McDougal, Phytochem., 1985, 24(1), 71; (b) D.M.W. Anderson and F.J. McDougal, Phytochem., 1985, 24(6), 1237; (c) D.M.W. Anderson, P.C. Bell and F.J. McDougal, Food Addit. Contam., 1986, 3(4), 305; (d) D.M.W. Anderson and F.J. McDougal, Food Addit. Contam., 1987, 4(2), 125,; (e) D.M.W. Anderson and F.J. McDougal, Food Addit. Contam., 1987(a), in press; (f) D.M.W. Anderson and F.J. McDougal, Food Addit. Contam., 1987(b), in press.

Some studies to which I contributed during this period of study, but not included in this Ph.D. Thesis, have been published and are presented in reprint form e.g. A.W.J. Anderson, W.J. Brydon, M.A. Eastwood, F.J. McDougal and D.M.W. Anderson, Food Addit. Contam., 1985, 2(1), 33; D.M.W. Anderson, P.C. Bell, M.C.L. Gill, F.J. McDougal and C.G.A. McNab, Phytochem., 1986, 25(1), 247; D.M.W. Anderson, F.J. McDougal and C.G.A. McNab, Food Hydrocolloids, 1987, 1(3), 243.

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ABSTRACT

Two commercial samples of gum arabic were characterised. The first, "gum arabic (old)", corresponded to analytical parameters for Acacia senegal gum. The other, "gum arabic (new)", was suspected to contain 10% of Acacia seyal gum.

A series of studies on laboratory rats were carried out involving gum arabic (new). At a level of 15% of the diet, gum arabic was found to cause caecal enlargement within 72 hours, mainly due to an increase in the % moisture content of the caecum. Caecal enlargement was reversible, again within 72 hours. Heat-treated gum arabic caused a similar response. Treatment of rats, receiving 15% gum arabic, with Metronidazole had no discernible effect. An antibiotic cocktail of Bacitracin, Neomycin Sulphate and Streptomycin greatly affected the digestion of gum arabic. Caecal enlargement was greatly increased. This increase appeared due to remnants of gum arabic in the caecal contents. Some bacterial populations in the caecum were eliminated, volatile fatty acid (V.F.A.) concentrations also diminished, indicating that fermentation of gum arabic to V.F.A.'s in the caecum had been prevented.

Solutions of gum arabic (new) were subjected to autohydrolysis, mild acidic hydrolysis and ultraviolet irradiation. In each case, three products were isolated:- an insoluble, highly proteinaceous precipitate; a degraded proteinaceous gum polysaccharide; and a diffusate. The results confirm the lability of some of the arabinose, rhamnose and galactose, and establish that some amino acids in gum arabic are more labile than others.

Data are presented for the amino acid and sugar compositions of gum arabic (old) and its four Smith-degradation products (0.34, 0.56, 0.87, 0.90 and 0.85%N respectively). The proteinaceous enrichment of the branched, galactan core largely involves serine, threonine and proline; this correlates well with previous degradative studies. Serine, threonine and proline are commonly encountered in sugar-amino acid linkages. Gums must now be regarded as proteoglycans and the amino acids must be taken into account.

Sixteen gum exudates of the Series Phyllodineae, Gummiferae and Vulgares were analysed for their polysaccharide and amino acid parameters. A further nine, previously characterised Acacia gum exudates, were analysed for their amino acid contents. It appears likely that the proteinaceous component is of structural importance even in those species with very low nitrogen contents.

Solutions of the gums from Acacia gerrardii, A. goetzei, A. eriopoda and A. tumida have been subjected to mild acidic hydrolysis; A. eriopoda was also subjected to ultraviolet irradiation. Data are presented for the sugar and amino acid compositions of the three products obtained from each gum. As in gum arabic, some amino acids are more labile than others; high proportions of serine and hydroxyproline in the natural gums become even higher in the degraded gums.

Amino acid data are presented for gum exudates from four Combretum species and for the degradation products of Combretum nigricans and Prosopis alba when subjected to mild acidic hydrolysis and ultraviolet irradiation. The results for the Prosopis gum (but not for the Combretum gum), closely resemble

those of the *Acacia* gums. Such gums are not permitted, world-wide, as food additives or ingredients and this data will facilitate their detection for legal purposes.

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CHAPTER I

GENERAL INTRODUCTION

The term 'gum' designates a great variety of natural products in the form of tears, flakes and angular fragments, sticky in nature, and found on the surface of trees. The plant gums have previously been regarded as complex, acidic hetero-polysaccharides, exuded from the stems of certain tropical and sub-tropical trees and shrubs found in Africa, Australia, India, South America and parts of Asia, but this thesis shows that at least some gums are more correctly regarded as proteoglycans. Exudation usually follows mechanical injury or bacterial infestation of the bark (1), but the precise mechanism of gum formation is still not fully understood; bacterial action, enzymic conversion of starch or hemicelluloses, and direct synthesis, have been mentioned as possible explanations (1). Functionally, plant gums may act to seal off wounds against further attack and also to form a protection against tissue dehydration.

Gums come from a variety of plants; utilisation of the natural water soluble gums was increasing at a rate of 10% per annum in the seventies. But regulatory decisions, economic and political factors and seasonal changes in weather conditions all influence the types of gum that are used, and the amounts of some gums (e.g. gums arabic, karaya and tragacanth) now being used are much less than in 1980. Labour cost escalation, even in remote areas, and the quality control needed to ensure relatively pure and constant quality material to meet modern food manufacturing standards causes some plant sources to be eliminated and others, perhaps more amenable to cultivation, to appear as new sources (2).

The gums occur as mixed salts of partially neutralised complex polysaccharide acids containing hexose, pentose, methylpentose and uronic acid residues linked together in a complex, highly branched manner. The neutral sugars most commonly found are D-galactose, L-arabinose and L-rhamnose, with D-xylose, D-mannose and D-glucose also present in certain species. The acidity of plant gums arises most frequently from the presence of D-glucuronic acid and its 4-O-methyl derivative, but some genera also contain D-galacturonic acid (1). Some exudate gums (e.g. gum arabic) are highly soluble in water, yielding viscous solutions; others (e.g. gums karaya, tragacanth) imbibe water to give suspensions or dissolve to a small extent e.g. 1-2%.

A small proportion (under 5%) of proteinaceous material is also present in most gums, although many species contain much higher levels, up to as much as 53% in Acacia difficilis gum (3). Evidence available to date seems to imply that there is chemical bonding between protein and polysaccharide i.e. plant gums are actually forms of proteoglycans. Attempts to isolate the proteinaceous material without causing extensive degradation to the polysaccharide have been unsuccessful (4,5).

The ability of some gums to dissolve readily to give viscous solutions makes them useful commercially (1,6,7). They are widely used in medicines, foods, cosmetics, adhesives, paints, inks, textiles etc. (8). They are excellent suspending, dispersing, stabilising, emulsifying, and gel-forming agents. They are also used as coagulants, binders, lubricants and film-formers.

However, the main use of plant gums is in the food industry where they are used as food additives and ingredients. Among the leading materials, in decreasing order of use in food are, pectins, gum arabic, alginates, guar gum, carboxymethylcellulose, carrageenan, locust bean gum and modified starches (9). It is now rare for a processed food not to contain a gum product (i) to correct or minimise defects in its natural ingredients, (ii) to increase the sensory satisfaction derived and (iii) to produce formulations that make possible new combinations of food ingredients (10,11).

Chapter III of this thesis presents studies on gum arabic, also known as gum acacia, which is mainly the exudate from the species Acacia senegal (syn. verek); good quality samples are practically colourless, odourless and flavourless and dissolve completely in water to give a stabilising solution of a useful viscosity ($[\eta]$ ca. 20 $ml g^{-1}$), a rare combination of desirable properties in a natural product.

The essential property of a food additive is, of course, a total lack of toxicity and because gum arabic has been used for centuries without any apparent ill effects, it has long been assumed to be safe. In the light, however, of increasingly responsible attitudes towards food additives and since their types and uses were steadily growing, the safety of gum arabic and other gums as food additives became a matter of concern. Ideally, an additive and its metabolites should be shown to be non-toxic, non-carcinogenic and non-allergenic, both when ingested in small quantities, and after long term exposure. After undergoing many trials, gum arabic has been

assessed toxicologically as a safe foodstuffs additive to which the "not specified" category of Acceptable Daily Intake (A.D.I.) was assigned in 1982 (12).

Two bulk, commercial samples of gum arabic were fully characterised in this study. One sample was found to correspond closely to analytical parameters for reputable, Sudanese commercial samples and also for authenticated, hand-picked specimens of Acacia senegal gum (13); the other sample is suspected to contain ca. 10% of the exudate from A. seyal (gum talha), causing a change in the specific rotation of the sample. This sample is representative of the quality of shipments of gum arabic supplied to the food industry during recent years of shortage and, as such, was used as the Test Article for feeding trials with laboratory rats, the results of which are presented in Chapter III. This Chapter also presents degradative studies of this sample using auto-hydrolysis, mild acidic hydrolysis and ultraviolet irradiation. In each case, three products were isolated:- an insoluble, highly proteinaceous precipitate; a degraded proteinaceous gum polysaccharide; and mixtures of mono- and di-saccharides etc. plus amino acids/peptides recovered from the dialysates of the treated solutions.

The superior gum arabic sample was subjected to sequential Smith-degradation; its four sequential products were analysed for their amino acid composition and quantitative sugar-amino acid relationships. Overall, 94% of the original sugars but only 85% of the amino acids were eliminated. The proteinaceous enrichment of the galactan core (0.34 to 0.85%N) largely involved serine, threonine and proline, although hydroxy-proline remained as the major amino acid present (ca. 30%).

This enrichment shows good correlation with that found in previous, less extensive degradation studies using auto-hydrolysis, mild acidic hydrolysis and ultraviolet irradiation. Serine, threonine, and proline are commonly encountered in sugar-amino acid linkages in glycoproteins and glycosamino-glycans.

The analytical parameters used to characterise gums are ash, protein and methoxyl contents, specific rotation, intrinsic viscosity, molecular and equivalent weights, uronic anhydride content, and neutral sugars composition after hydrolysis. These parameters, taken overall, establish a form of "fingerprint", which is characteristic of the gum from each particular botanical species. Now, in addition, the amino acid composition can be used to complement the combination of parameters of a gum from any genus (Acacia, Albizia, Araucaria, Combretum, Grevillea, Parkia, Prosopis etc.). This gives one of the most sensitive ways of establishing the identity of a botanical species (7). Consequently, a great deal of analytical work has been done to obtain data to support chemotaxonomy, especially in the genus Acacia, the most studied and commercially the most important gum-bearing genus.

Bentham, in 1875, classified the genus Acacia into six Series (14), a classification which has largely been supported by chemical analysis to date. Chapter IV of this thesis reports an analytical study of fourteen Acacia species belonging to Bentham's Series 1. Phyllodineae, sub-series Uninerves racemosae and one member of sub-series Plurinerves racemosae, none of which have been characterised previously. Amino acid

compositions for most of these samples and for a further five members of Uninerves racemosae and three members of sub-series Juliflorae are also reported. In addition, analytical data for the sugar and amino acid compositions are presented for the gum exudates from Acacia gerrardii (Series 4. Gummiferae) and Acacia goetzei (Series 5. Vulgares), which likewise have not been studied previously. Amino acid compositions are also reported for A. deanei (Series 2. Botryocephalae) and A. xanthophloea (Series 4. Gummiferae).

Chapter IV also presents a degradative study of some of the more highly proteinaceous of these Acacia gum exudates. Solutions of the gums from Acacia gerrardii, A. goetzei, and A. tumida (%N = 1.86, 0.89 and 7.66 respectively) were subjected to mild acidic hydrolysis; a solution of the gum from A. eriopoda (%N = 6.70) was subjected to ultraviolet irradiation and acidic hydrolysis. Data are presented for the sugar and amino acid compositions of the three products obtained from each gum by the degradative processes used. The results extend those obtained previously for gum arabic; acidic hydrolysis and ultraviolet irradiation gave differing yields of the insoluble and diffusate fractions having different analytical parameters.

Chapter V of this thesis presents the amino acid compositions of four members of the genus Combretum and reports the fate of the amino acids present in samples of the gums from Combretum nigricans and Prosopis alba when subjected to mild acidic hydrolysis and ultraviolet irradiation. Both Combretum and Prosopis gums have been used in the past as adulterants of, or substitutes for, gum arabic, although neither are

included in the American G.R.A.S. list nor in any other list of permitted food additives.

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CHAPTER II

EXPERIMENTAL METHODS

II.1. GENERAL METHODS

Weighings. All accurate weighings were made within the range of the graticule scale (range 0-100mg) of a Stanton Unimatic Model C.L.1. single-pan balance, having an accuracy of ±0.1mg.

Dialyses of polysaccharides, to remove low molecular weight material, were carried out in visking cellophane tubing (MEDICELL International Ltd., London) against running tap water for 48-72 hours unless otherwise stated.

Electrodialyses of polysaccharides were carried out in a three-compartment perspex cell fitted with cellophane membranes. The water in the outer electrode compartments was changed regularly to prevent overheating. Electrodialysis (applied voltage = 300 volts) was continued until a current ceased to flow.

Reductions in volume were carried out with a rotary evaporator at temperatures below 40°C, unless otherwise stated.

Moisture contents were determined by heating to constant weight at 105°C.

Ash contents were determined by heating to constant weight in a muffle furnace at 550°C.

Carbon, hydrogen and nitrogen contents were determined with a Carlo-Erba mod.1106 Elemental Analyser. Nitrogen contents were also determined by a semi-micro Kjeldahl method.

Methoxyl contents were determined by a vapour phase infrared method (1,2); a calibration curve was based on known weights

of methyl iodide. Infrared spectroscopy was carried out with a Perkin-Elmer 137 spectrophotometer.

Equivalent weight determinations on exhaustively electrodialysed polysaccharides were carried out by direct titration with standard sodium hydroxide solution (ca. 0.01N).

Uronic acid contents were calculated from the equivalent weights as (17,600/E.W.), i.e. values are expressed as uronic anhydride.

Quantitative estimation of sugars. Sugars were separated from hydrolysates by chromatography in solvents (b) or (d) on Whatman 3MM papers. After elution from the paper in boiling water, sugars were estimated colourimetrically by the phenol-sulphuric acid method (3). The optical density was read on an Unicam SP 1300 spectrophotometer using filter 2. Calibration curves were obtained from known weights of sugars.

II.2. PHYSICAL METHODS

Specific rotations of aqueous solutions were measured using the sodium D-line with a Perkin-Elmer Model 141 polarimeter at 20±2°C. All solutions were first clarified by passage through filters of average pore size 0.8μm (Millipore Ltd., Bedford, Mass., U.S.A.) with a stainless steel filter holder attached to a syringe (20ml). Concentrations of gum were assumed to be unaltered by filtration (5)

Viscosity determinations were carried out in M-sodium chloride solution in an Ubbelohde suspended-level dilution

viscometer at 25.0±0.1°C. Solutions were filtered carefully before additions were made to the viscometer. Flow times were measured to within 0.1 second by means of a stop watch. The isoionic dilution technique was used; a solution of the gum (6ml, 1-2%) was placed in the viscometer and the flow time measured. Flow times were also obtained for successive dilutions with M-sodium chloride solutions (four additions of 2ml each). Since preliminary experiments had indicated that any loss of gum from M-sodium chloride solution on filtering was negligible, concentration values were estimated from the dry weight of gum dissolved in a known volume.

Assuming the densities of M-sodium chloride and gum solutions to be equal for low concentration of gum, the intrinsic viscosity number $[\eta]$, is given by

$$[\eta] = \lim_{c \to o} \frac{\eta sp}{c} = \lim_{c \to o} \frac{t - t_o}{c t_o}$$

where c is the concentration of gum (g/ml) and t_o and t are the flow times (seconds) for solvent and solution respectively. Linear extrapolation of the plot of $\frac{t-t_o}{ct_o}$ against c to t=0 gives $[\eta]$ (4).

Light scattering measurements, for molecular weight determinations, were carried out at 28±0.5°C with a SOFICA photogoniodiffusometer Model 4200. Unpolarised green light (546nm) was selected from a mercury lamp spectrum with a Wratten Kodak N61 filter. Using the limiting viscosity number to give an indication of a suitable concentration, and using M-sodium chloride as solvent, gum solutions were accurately prepared (0.1-0.4g in 50ml). Dilutions of this solution were made;

the molecular weight was calculated as an average of three of these solutions. The solutions were clarified and made dust-free by passage through filters of average pore size 0.8μm and then through filters of average pore size 0.22μm (Millipore Ltd., Bedford, Mass., U.S.A.), using a stainless steel filter holder attached to a 20 ml syringe. Concentrations of gum solutions were assumed to be unaltered by ultrafiltration (5).

For each concentration, the intensity of scattered light at various angles between 30° and 150° was recorded and corrected; corrected scale readings I_θ for angle θ were calculated (6) from the equation:

$$I_\theta = \frac{(I_{soln.} - I_{sol.})\sin\theta}{1 + \cos^2\theta}$$

where $I_{soln.}$ and I_{sol} are the scale readings for polymer solution and solvent respectively. The reciprocal corrected scale reading $1/I_\theta$ is plotted against $\frac{\sin^2\theta}{2}$. Extrapolation of the linear portion of this graph to θ = 0 gives a value for $[1/I_\theta]_{\theta=0}$. The downward curvature of these graphs at low angles is thought to be caused by dust particles suspended in solution (7).

Molecular weights are then calculated from the equation:

$$M = \frac{R}{\frac{2\pi^2 n_o^2}{\lambda^4 N} \cdot [dn/dc]^2 \cdot I_B \cdot c.[1/I_\theta]_{\theta=0}}$$

where n_o = refractive index of solvent (1.340)

n = refractive index of solution

N = Avogadro's number (6.023×10^{23})

λ = wavelength of incident light (546nm)

c = concentration in g/ml

I_B = intensity diffused, selected for standard benzene (0.5)

dn/dc = refractive index increment

R = Rayleigh's constant (16.3×10^{-6})

Using the value for dn/dc of 0.146 (the average value found (8) for a series of Acacia gums) the above equation is simplified to:

$$M = \frac{2.309 \times 10^2}{c[1/I_\theta]_{\theta=0}}$$

II.3. CHEMICAL METHODS

Small scale polysaccharide hydrolyses were carried out overnight with N-sulphuric acid on a boiling water bath, unless otherwise stated. Hydrolysates were neutralised with barium carbonate, filtered, deionised with Amberlite IR-120(H) resin, and concentrated to a syrup on a rotary evaporator.

Periodate oxidations of polysaccharides were carried out in darkness at room temperature.

The formic acid released was estimated titrimetrically (9) with standard sodium hydroxide (ca. 0.01N) for portions (1 ml) of the solution. Methyl red was used as the indicator.

Amino acid hydrolysis. A sufficient amount of sample to give 2 mg of nitrogen (12.5 mg crude protein) was weighed and transferred quantitatively to a 100 ml round-bottomed, two-necked flask. Anti-bumping granules, 80 ml 6N hydrochloric acid and 2 ml 0.004M nor-leucine were added and the flask fitted to an 800 mm air-cooled condenser. The apparatus was purged with oxygen-free nitrogen and the contents heated under reflux for

20 hours under a continuous and slow stream of oxygen-free nitrogen. The solution was filtered through Whatman No 42 filter paper and evaporated to dryness at 42°C. The residue was dissolved in 20 ml 0.01N hydrochloric acid, filtered through 0.22µm Millipore filter and stored frozen in glass vials pending analysis.

Analysis was effected on a Rank Hilger Chromaspek as follows:

A suitable aliquot (normally 50µl) is applied to a 350x3 mm stainless steel column of cationic exchange resin (6µ beads from Rank Hilger) and the constituent amino acids separated at high pressure (ca. 2,000 lbs/in^2) by elution with lithium citrate buffers of increasing ionic strength and pH. The eluted amino acids are detected by reaction with ninhydrin in a continuous flow analytical system and quantified by references to standard solutions at 570 nm (440 nm for proline and hydroxyproline).

II.4 CHROMATOGRAPHIC SEPARATIONS

Paper chromatography of sugars was carried out on Whatman No. 1 papers, unless otherwise stated, with the following solvent systems (v/v):

a) ethyl acetate, acetic acid, formic acid, water (18:3:1:4)

b) benzene, butan-1-ol, pyridine, water (1:5:3:3, upper layer)

c) ethanol, hydrochloric acid (0.1N), butan-1-ol (10:5:1) (10)

d) ethyl acetate, pyridine, water (10:4:3).

Before using solvent system (c), papers were dipped in 0.3M-sodium dihydrogen orthophosphate solution and air dried.

Reducing sugars were detected by spraying chromatograms with a saturated solution of aniline oxalate in ethanol/water (1:1, v/v), then heating at 150°C for ca. 3 minutes.

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CHAPTER III

STUDIES ON GUM ARABIC (*ACACIA SENEGAL*)

CHAPTER III.1. INTRODUCTION

Gum arabic is defined as "a dried gummy exudate obtained from stems and branches of Acacia senegal (L.) Willd. or of related species of Acacia" (1) and, as such, is a permitted foodstuffs additive within the EEC (E414). The definition adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is essentially similar (2). Taxonomically, only Acacia laeta, A. mellifera, A. polyacantha and comparatively rare species within the "Acacia senegal complex" (3) are admissible as related species of A. senegal; because such species occur only sporadically and are not intensively cultivated nor tapped commercially within the Sahel, their gums are not available commercially as separate commodities for blending or other purposes. Accordingly Acacia senegal, the tree exclusively cultivated within the Sudan for gum-producing purposes, is now acknowledged internationally (4,5,6,7) as the only botanical source of gum arabic of practical commercial importance. Acacia trees are rarely greater than 8-10 feet tall and are uniquely adapted for survival under the most arid conditions.

Gum arabic has a highly branched structure and is the half-neutralised salt of an acidic polysaccharide, arabic acid, containing calcium, magnesium, sodium, potassium and other cations (e.g. iron, manganese, zinc). Complete hydrolysis with dilute acid yields D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. In addition to neutral sugars and uronic acid, analysis has revealed protein and hexosamine as components of gum arabic (8). The present specifications,

therefore, could be further improved and extended by the description of gum arabic as a proteinaceous polysaccharide, with reference to typical values for nitrogen content and its amino acid composition. Within the polysaccharide field, gum arabic is unique in having high solubility, forming aqueous solutions up to a concentration of 50% (w/v).

Commercially, good quality gum arabic (i.e. clean, good solubility in water, giving colourless or pale yellow solutions) is used in the pharmaceutical, cosmetic and food industries, whereas inferior grades (darker in colour or less readily soluble) are used in lithography, paints and inks, foundry sands and ceramics etc.

The main use of gum arabic is in the food industry, due to its properties as an emulsifier, stabiliser and thickener (9). It is used to influence the viscosity, body and texture of foods and imparts certain properties to food that cannot be obtained from other types of materials. It is odourless, colourless, tasteless and completely water-soluble and does not affect the flavour, colour or odour of other food ingredients. Gum arabic has been assessed toxicologically as a safe foodstuffs additive to which the "without limit" category of Acceptable Daily Intake (A.D.I.) was assigned in 1982 (10). Thus, it is important to have precise specifications for the purity and identity of gum arabic for trade and enforcement purposes, as there are no toxicological data of any kind for any Acacia gum other than that from Acacia senegal.

There are many uses to which gum arabic is put in the food industry. Some examples are:- it prevents ice crystal formation by absorbing and binding water; it emulsifies, thus

causing the uniform distribution of fat throughout a product; and it is used as an adhesive in glazes and toppings. In the cosmetic industry gum arabic is used in lotions and protective creams as it stabilises emulsions and increases their viscosities. In the pharmaceutical industry, gum arabic acts as a binder in tablets and pills and is also used in laxatives and to suspend poorly soluble, medicinal substances e.g. fat-soluble vitamins.

CHAPTER III.2. CHARACTERISATION OF COMMERCIAL GUM ARABIC SAMPLES USED AS TEST ARTICLES

III.2(i) INTRODUCTION

Large commercial consignments of gum arabic contain many gum collections, possibly from a range of provenances. For such a complex natural product, which is subject to seasonal and also geographical variations in composition (11), all that can be done to satisfy modern demands for specifications of identity and purity is to establish the range and average values for each analytical parameter from data for an adequate number of representative samples. This has been done for the polysaccharide parameters (12) and amino acid compositions (13) of gum arabic.

In this study, a commercial sample, "gum arabic (old)" used previously, and a second commercial sample, "gum arabic (new)", were characterized completely. It was important to establish that these Test Articles were representative samples of fair average quality before using them as a basis for further study.

III.2(ii) ORIGIN OF SAMPLES

Both commercial samples of gum were kindly provided by Rowntree Mackintosh Ltd., York, and had been recovered by spray-drying from a production batch of confectionery gum liquor prepared by dissolving commercial gum arabic in hot water (33% w/v) followed by processes to remove contaminating bark or sand etc. Crude gum arabic must be cleaned before use in foods (10). 100 kg of gum arabic (old) and 60 kg of gum arabic (new) were received, these amounts having been amassed over a period of several months from weekly samples tested for quality control purposes; the Test Articles were, therefore, representative of large tonnages of food-grade gum arabic.

III.2(iii) RESULTS AND DISCUSSION

The data in Table III.1 shows that gum arabic (old) has analytical parameters that correspond closely with those for reputable commercial samples of gum arabic from the main producing countries. It also corresponds closely with those for authenticated, hand-picked, specimens of *Acacia senegal* gum (12). While gum arabic (new) is also closely similar, one of its parameters, the specific rotation ($[\alpha]_D = -20°$), differs considerably. It has been stated (12) that "samples of gum arabic with specific rotations more positive than -25° or -26° must always be suspected to contain *Acacia seyal*, *A. drepanolobium*, or other unrelated gums in admixture with *A. senegal*." *A. seyal* and *A. drepanolobium* are the only other two *Acacia* gums marketed in commercial quantities and have

TABLE III.1 Analytical data for commerical samples of gum arabic

	6 (12)	7 (12)	8 (12)	9 (12)	10 (12)	11 (12)	12 (12)	gum arabic (old)	gum arabic (new)
Moisture, %	13.5	13.1	13.6	16.4	16.4	13.6	13.3	10.2	5.0
Ash, %[a]	3.6	3.8	3.9	4.3	4.4	4.1	4.9	3.5	3.6
Nitrogen, %[a]	0.58	0.28	0.46	0.35	0.32	0.32	0.39	0.39	0.34
Nitrogen conversion factor (NCF)	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.76	6.43
Hence protein, %(NxNCF)[a]	3.6	1.7	2.9	2.2	2.0	2.0	2.4	2.64	2.19
Methoxyl, %[b]	0.18	0.14	0.26	0.20	0.14	0.37	0.52	0.32	0.39
Specific rotation in water (degrees)[b]	-32	-29	-31	-33	-32	-32	-29	-28	-20
Intrinsic viscosity, mlg^{-1}[a]	20	19	17	19	17	17	18	16	15
Molecular weight, ($M^w \times 10^5$)[a]	6.4	11	8.3	6.6	5.0	4.8	5.1	7.5	7.6
Equivalent weight[b]	1,270	970	1000	870	990	860	890	1020	982
Hence uronic anhydride, %[bc]	14	18	18	20	18	20	20	17	18
Sugar composition after hydrolysis (%):-									
4-O-Methyl glucuronic acid	1	1	1.5	1	1	2	3	1.5	2
Glucuronic acid	13	17	16.5	19	17	18	17	15.5	16
Galactose	46	46	45	43	46	44	46	45	48
Arabinose	27	26	27	21	22	21	22	24	25
Rhamnose	13	10	10	16	14	15	12	14	9

Notes:
[a] corrected for moisture content
[b] corrected for moisture and protein content
[c] if all acidity arises from uronic acids
[d] if all methoxyl groups located in this acid

analytical parameters (14,15) which differ greatly from those of A. senegal gum and are readily distinguishable on account of their highly positive specific rotations. In all published botanical classifications and revisions of the genus Acacia, A. seyal and A. drepanolobium are assigned to a different sub-division from A. senegal and thus cannot be regarded as botanically related species. Reference to a definite value for the quite strongly negative specific rotation of true gum arabic (e.g. Table III.1) rather than to the much less precise present reference to "slightly laevorotatory solutions" (2) would greatly strengthen the present specification for identity and purity for trade and enforcement purposes. In this instance, it is suggested that a 10% substitution of the commercially cheaper A. seyal could cause the change in rotation of gum arabic (new), without significantly altering any of the other parameters.

The amino acid compositions reported in Table III.2 show that there are differences between the samples to an extent comparable with that long established (9) for the polysaccharide parameters of all Acacia gum exudates. Despite these natural variations, the proteinaceous component of gum arabic is clearly characterised by the presence of large proportions of hydroxyproline (ca. 30% of the amino acids present) and serine (ca. 14%). These amino acids have long been known to be involved in covalent linkages between the polysaccharide and protein moieties in glycoproteins and proteoglycans (16,17,18) and may, therefore, be of structural significance (8). The role of the proteinaceous component has not yet been taken into account in structural considerations

TABLE III.2 Amino acid composition of commercial samples of gum arabic

	1 (13)	2 (13)	gum arabic (old) 3 (13)	4 (13)	gum arabic (new) 5 (13)	6 (13)	7 (13)
% Nitrogen	0.31	0.29	~~0.23~~ 0.39	0.58	0.28	0.46	0.31
Alanine	30	32	31	30	32	33	31
Arginine	11	9	7	6	6	7	6
Aspartic acid	62	59	63	55	63	59	65
Cystine	0	0	0	1	1	1	0
Glutamic acid	39	36	35	32	35	36	43
Glycine	62	56	48	42	44	46	48
Histidine	58	51	39	44	73	47	40
Hydroxyproline	233	228	313	319	262	292	282
Isoleucine	15	16	13	13	14	12	12
Leucine	85	85	73	68	72	67	71
Lysine	31	29	25	23	22	25	25
Methionine	0	0	1	0	1	1	0
Phenylalanine	37	31	27	25	27	27	33
Proline	72	74	81	83	71	86	84
Serine	134	153	111	137	143	137	126
Threonine	74	83	79	69	77	70	76
Tyrosine	13	11	10	10	11	12	9
Valine	44	46	45	43	47	44	48

of gum arabic molecules (5,6,19). The amino acid composition of A. seyal is essentially similar to that of gum arabic (unpublished results), and therefore its presence or absence in the samples, gum arabic (new), could not be detected in this manner.

CHAPTER III.3. LABORATORY RAT FEEDING TRIALS WITH TEST ARTICLE "GUM ARABIC (NEW)"

III.3(i) INTRODUCTION

Gum arabic is a type of soluble dietary fibre, unusual in that it is distinct from the cereal-bran type of fibre. Dietary fibre was defined by Trowell in 1974 (20) as "that part of plant material in our diet which is resistant to digestion by secretions of the human digestive tract". As this definition did not include polysaccharides present in some food additives (such as plant gums, algal polysaccharides, pectins, modified celluloses, and modified starches), Trowell et al. (21) extended the definition to include all the polysaccharides and lignin that are not digested by endogenous secretions of the human digestive tract. Although most of the dietary fibre constituents may survive digestion in the mouth, stomach and small intestine, some of the constituents may be degraded by micro-organisms of the human colon (22,23).

The gums have been defined technically by one group of workers (24) as polymeric materials that can be dissolved or dispersed in water to give a thickening and/or a gelling effect. Among the leading materials, in decreasing order of use in food, are pectins, gum arabic, alginates, guar gum,

carboxymethylcellulose, carrageenan, locust bean gum, and modified starches (25). These gums have valuable properties; many commercial foods contain small amounts of them. After a first period of enthusiasm, gum manufacturers are now reluctant to promote their products as dietary fibre, because of warnings from regulatory agencies that claims for the cholesterol lowering and other effects may cause these products to be considered as drugs (26). However, dietary fibre promotion and consumption, and dietary fibre research, are still important factors in today's food industry. Research activities involve two approaches - chemical structure/ physiological action relationships, and formulation of palatable foods with high fibre content.

Fibre has been reported to have diverse effects on function along the gastrointestinal tract, influencing gastric emptying time, intestinal absorption, caecal fermentation and faecal weight (27). It appears to influence each of these functions in a manner peculiar to the fibre source, for example plant hydrocolloids such as pectin and gum arabic have a strong retarding action on digesta transit, whereas another group of hydrocolloids e.g. guar, carrageenan, tragacanth and carboxymethylcellulose, accelerated digesta passage to various degrees. It is reported (28) that this effect of hydrocolloids on digesta transit is probably not purely physical; in animal feeding studies there is a long delay, after the animals have been returned to normal feed, before the digesta transit time corresponds with that of a control group. However, it has been shown(29) that the viscosity of a gum is an indicator of its effect on gastric

emptying. It is also thought that the viscosity enhancing property of gums is responsible for a lengthening of the small intestine in rats after being fed dose levels of 10-40% of e.g. gum arabic, tragacanth, carrageenan, gum karaya, methylcellulose (30). By means of an unstirred layer resistance (31,32) more nutrients reach distal parts of the small intestine and this may serve as a stimulus for the longitudinal growth response.

Little is known about the immunological properties of exudate gums, and whether or not they have the potential to contribute to food-related hypersensitivity diseases. They may occasionally induce allergy in skin or lungs and have been shown to be capable of inducing active systemic immune responses, comparable to, but not greater than, that elicited by a protein antigen e.g. hen's egg ovalbumin, when injected into the footpads of mice (38). However, in other experiments, the specific immune responses of the mouse to gum arabic when given by the natural, oral route have been examined (39), and this has been shown to induce the protective response of oral tolerance. Further studies (40) confirm that the exudate gums arabic, karaya and tragacanth are capable of inducing immune reactions when introduced into the tissues of mice, i.e. are antigenic. In addition, some of the preparations, although of food grade and complying with various national and international regulations, have non-specific, non-immunologically-based irritant effects when injected. Samples of gums obtained from different countries are antigenically similar, and processing of gum arabic to effect removal of surface contaminants has little or no effect on its

inherent immunogenicity. No attempt was made to establish whether the relevant antigenic determinants were confined to the polysaccharide components of the gum molecules or whether the small amounts of proteinaceous matter present in gums is important in antigenicity. However, these immune responses to the gums are similar to immune responses to other harmless foodstuffs.

Many of the earlier studies on gum arabic were mutually contradictory and none involved modern standards of testing. The need to evaluate the health aspects of gum arabic as a food ingredient has however ensured continuing interest and research in this field. Recent studies (33) involving two independent teams of specialists have not revealed any abnormalities in the ultrastructure of heart and liver tissues of rats fed with the highest advisable (34) dietary levels of gum arabic and gum tragacanth. The results do not support earlier suggestions, based on in vitro assays, that gum arabic and gum tragacanth impaired mitochondrial function (35,36); however, they do confirm a previous finding (37), that microsomal P-450 values were unchanged.

Although it was previously reported (41) that the administration of 25 g of gum arabic (Acacia senegal) daily for 25 days had little effect on glucose tolerance, the gum from Acacia arabica has recently been shown (42) to improve glucose and insulin tolerance in normal subjects when consumed in a moderate dose (20 g) along with glucose (100 g) in a glucose tolerance test. This response was found to be less marked than that of guar gum which has been shown to reduce post-prandial glucose and insulin levels in healthy volunteers

(29), and in diabetics (43). However, guar gum and other fibres, although effective as hypoglycemic agents, are very difficult to incorporate into palatable food products because of their high viscosity. These results (42) are only preliminary; it should not be assumed that a decrease in glucose and insulin during glucose tolerance testing with gum arabic in healthy non-diabetic subjects, will result in improved glucose and insulin response in diabetics.

A study of the effect of gum arabic in humans (41) indicated that the administration of 25 g of gum arabic daily for 21 days had little effect on stool weight. Serum cholesterol decreased marginally but the mechanism of the decrease was not by adsorption of cholesterol metabolites, as the faecal bile acids and neutral sterols did not increase. The absence of precipitable gum arabic in the faeces of the subjects and the marked increases in breath hydrogen production would indicate bacterial breakdown of the polysaccharide in the human colon after three week administration of gum arabic. This was in contrast with acute administration experiments (41) where breath hydrogen did not increase, and therefore suggests either bacterial proliferation or increased metabolism. It seems, therefore, that gum arabic, when ingested in a relatively large dose (although this is comparable to those used when administering other fibre sources therapeutically) has no toxic effect on the basis of the tests used on the subjects in this experiment (41) and, furthermore, little systemic metabolic action of consequence. It seems clear that the molecule is degraded during its passage through the human colon and that bacterial action is

responsible for this. Bacteroides species account for about 20% of the human faecal flora, but other major genera such as Bifidobacterium, Peptostreptococcus, Lactobacillus, Ruminococcus, Coprococcus, Eubacterium and Fusobacterium are also present in relatively high concentrations in the human colon (44,45). Most of these species are saccharolytic, that is they derive their energy primarily from carbohydrate and its derivatives.

Many studies with laboratory rats have been carried out. Although care must be taken about extrapolating data from animal studies directly to human subjects, because rats have a functional caecum and humans do not, much useful information has been gained. A sub-chronic (90 day) toxicity test involving young Wistar rats fed gum arabic at dietary levels of 0% (two control groups), 1, 2, 4, 8 and 20% for 13 weeks was carried out (46). The criteria studied were body weights, food and water consumption, urinalysis, liver and kidney weights, clinical chemistry, haematology and histology. No untoward effects were observed at dose levels below those which caused dietary imbalance. At the top dose, female rats showed a small reduction in kidney weight, caecal enlargement, and changes in the serum urea and total CO_2. Male rats showed no differences from the control groups at dietary concentrations up to approximately 8%, but food and water consumption, body weight, liver and kidney weights, all decreased significantly and caecal enlargement was evident at the top dose tested. There were no histological changes and no significant changes in haematological parameters in male or female rats at the top dose tested. The no-untoward

effect concentrations were 8.6% (5.2 g/kg/day) and 18.1% (13.8 g/kg/day) for male and female rats respectively. A two year carcinogenesis bio-assay (47) also showed that gum arabic does not act as a carcinogen in rats and mice of either sex, and no other compound-related clinical signs or effects on survival were noted. The feeding diets used contained 25,000 p.p.m. or 50,000 p.p.m. of gum arabic.

Since these studies led to a conclusion that gum arabic has no untoward effect in rats at dietary concentrations below that (8%) which produces a nutritional imbalance, further studies focussed interest on the effect of gum arabic at high dietary levels on caecal enlargement, and the role of caecal microflora in the complete fermentative decomposition of gum arabic in the rat. Experiments (48) using both a normal breeder's diet and a low-residue elemental diet showed that gum arabic could be recovered from the stomach to the small intestine, but not from the caecum, colon, or rectum of rats fed with gum arabic. Caecal excision and restoration of intestinal continuity resulted, however, in the recovery of gum arabic from stomach to rectum. It was therefore concluded that gum arabic is not significantly metabolised nor degraded in the stomach, but is rapidly decomposed by bacterial activity within the caecum, associated with increased breath methane production, increased concentrations of total volatile fatty acids, and changes in the relative proportions of the faecal volatile fatty acids. When gum arabic was given with the normal breeder's diet there was an increase in caecal volatile fatty acid concentrations, but when given with an elemental diet there was a

decrease. This may be due to a variation in production, bacterial utilisation or caecal absorption. It does suggest, however, that the congeners, the other constituents of the diet, affect the mode in which gum arabic is metabolised.

The rat, a single-stomached omnivore, is a suitable animal to feed with diets varying in composition for the study of bacterial metabolism in the caecum. The caecum is an organ containing a culture of mixed strains of many types of microorganisms. The metabolic activity of the caecum depends on the particular strains of microorganisms in the environment that manage to colonise the caecum and their relevant proportions, and these are likely to be determined by the nutrients available to them. Even were all of an animal's food digestible, the microorganisms would still have nutrients in the form of sloughed mucosal cells and digestive enzymes. Since no foods are completely digested, some of their components may provide extra nutrients for the microorganisms or simply dilute the caecal contents. It is unlikely that any counting procedure could accurately predict the metabolic activity of caecal mircoorganisms, which may alter rapidly when a new strain, able to compete with the others more effectively (49), appears.

Comparative studies in which the same dietary fibre preparations are tested both in rat- and human-balance experiments are needed to document the usefulness of the rat experimental model to predict the physiologically-important property of bacterial fermentability exhibited by dietary fibre (50). It seems likely that certain chemical interactions

in dietary fibre polymers, or between such polymers and other dietary components e.g. proteins, limit the bacterial degradation of certain kinds of dietary fibre. The structural differences between fermentable and non-fermentable dietary fibre needs further investigation.

The aim of this study, with small numbers of laboratory rats, was to get a clearer indication of the extent of caecal enlargement caused by gum arabic and its possible causes and effects, and to determine the timing and nature of this enlargement and its reversibility. Antibiotics were used in an attempt to change the bacterial population of the caecum to see if this had any effect on the digestion of gum arabic. Gum arabic, which had been heat-treated to denature its protein content, was also used to ascertain the extent, if any, to which this would alter the effect of the gum on the caecum. Gum arabic (new) was used in these feeding trials as, although it had been found to be not completely typical of Acacia senegal gum, it must be considered as representative of the grade of gum arabic more commonly used in the food industry.

III.3(ii) MATERIALS AND METHODS

All the animals used in the following experiments were male, albino, Wistar rats provided by the Animal Unit, Western General Hospital, Edinburgh. The animals were normally housed four or six per cage. The test article was gum arabic (new) as characterised in Chapter III.2. The control diet was a standard small animal diet, Labsure CRM(X), which had

been milled to a powder. Gum arabic was incorporated in specified amounts for the test animals. In all cases, water was added to the diet, or diet plus gum arabic, to produce a thick paste in order to minimise losses due to scattering.

In the first experiment twelve rats formed the control group (A), while the test group (B) of thirty-six rats received a supplement of 8% (w/w) gum arabic. Body weights were in the range 300-400 g. The rats were killed by ether inhalation at two to three day intervals and the caecal weight determined (Table III.3). After four days caecal enlargement was already apparent but the results were not conclusive, therefore from day 15 of the experiment, the gum arabic supplement was increased to 15% (w/w). From day 22 the test rats were split into two groups:- Group B - still receiving 15% gum arabic supplement; and Group C - receiving the control diet (as Group A).

% Dry matter determinations of caecal contents were made by oven-drying at 105°C and weights were obtained for caecal tissue after washing and blotting dry the caecal wall. On days 37 and 43 caecal contents were centrifuged using a 2LMSE Mistral centrifuge at 14,000G with an angle head rotor for 30 minutes. Aliquots of the supernatant solutions were analysed for osmotic pressure (Wolfson Lab., Western General Hospital). The remaining supernatant solutions were freeze-dried and analysed for sugar, nitrogen and amino acid contents (Table III.4).

Experiment 1 was repeated on a group of thirty-six rats, body weights 200-300 g, with eight rats forming a control

TABLE III.3 Caecal weight (g) per 100 g body weight for rats fed a control diet (group A) plus 8% increasing to 15% gum arabic (group B) and reversion (group C)

Day No.	Caecal weight (g) per 100 g body weight		
	Group A	Group B	Group C
4	1.18	1.55	
7	0.99	1.31	
9	1.57	1.90	
11		1.56	
14	1.55	1.42	
15		gum arabic supplement increased from 8% to 15%	
16	1.33	2.29, 1.73	
18		1.60	
21	1.17	2.16	
22		1.36	Group C off gum arabic
23		2.11	1.75
24		1.45	1.55
25		2.37	1.62
28	1.25	2.31	1.31
29		2.40	1.44
30		2.02	1.84
31		2.02	2.20
32		3.27	1.72
35	1.47	2.96	1.46
37	1.30	2.09	1.58
43	1.63, 1.42	3.09, 3.10	1.51, 1.75

TABLE III.4 Amino acid compositions of the caecal contents of group A and group B rats (Experiment 1) and of gum arabic (new)

	Group A Control	Group B Test	gum arabic (new)
% Nitrogen	3.87	4.49	0.36
Alanine	107	118	32
Arginine	trace	18	6
Aspartic acid	130	118	65
Cystine	8	6	1
Glutamic acid	127	123	35
Glycine	93	82	44
Histidine	70	48	73
Hydroxyproline	5	13	262
Isoleucine	36	44	14
Leucine	56	58	72
Lysine	52	60	22
Methionine	7	19	1
Phenylalanine	21	26	27
Proline	56	41	71
Serine	67	61	143
Threonine	76	71	77
Tyrosine	22	24	11
Valine	66	69	47

group (A) and thirty-two rats receiving gum arabic at 15% (w/w) (Group B). Rats were killed in pairs and caecal weights determined at 1, 2 and 3 day intervals over a period of two weeks (Table III.5). All but two test rats were returned to the control diet and the programme of necropsy continued as before. % Dry matter determinations and analysis of microfloral contents was carried out on the caecal contents of rats still receiving gum arabic (Group B), and on the caecal contents of rats no longer receiving gum arabic (Group C), and also on control (Group A) rats. It was hoped that duplicate results would eliminate some of the randomised results of experiment 1. The bacterial counts were carried out by the East of Scotland College of Agriculture.

In the third experiment thirty weanling rats, body weights 50-150 g, were split into three groups:- Group A (6 rats) - a control group; Group B (12 rats) - receiving a gum arabic supplement; and Group C (12 rats) - receiving a heat-treated gum arabic supplement. As the rats used were much smaller than in the previous two experiments, the supplements for groups B and C were introduced gradually, starting at 8% (w/w), increased to 12% (w/w) on day 5 of the trial and finally to a level of 16% (w/w) on day 9. The treated gum arabic had been heated at 105°C for a minimum of 24 hours, a process which impairs the solubility of the gum and denatures its protein content. Group C rats were examined for caecal enlargement (Table III.6), volatile fatty acid content (Table III.7) and reversibility after being taken off the supplement on day 64 of the trial. Once caecal enlargement had been established (Table III.6) and V.F.A. analysis carried

TABLE III.5 Caecal weight (g) per 100 g body weight for rats fed a control diet (group A) plus 15% gum arabic (group B) and reversion (group C)

Day No.	Caecal weight (g) per 100 g body weight		
	Group A	Group B	Group C
2	1.91	1.55, 2.11	
3		3.12, 3.35	
4	1.91	2.61, 3.09	
7		2.63, 3.04	
9	2.02	2.71, 2.78	
11		2.80, 3.37	
14	2.30	2.92, 3.50	
22			Group C off gum arabic
23			2.46, 2.63
24	1.60	2.67, 2.69	2.50, 2.95
25			1.85. 2.14
28	1.86		2.10, 2.34
35	1.95		1.87, 2.16
42	2.01		2.28, 2.60

TABLE III.6 Caecal weight (g) per 100 g body weight for rats fed a control diet (group A) plus 16% gum arabic (group B) receiving antibiotics (Bacitracin, Neomycin Sulphate and Streptomycin at 1 mg ml^{-1}) from day 77 and group C receiving heat treated gum arabic at 16% till day 64.

Day No.	Caecal weight (g) per 100 g body weight		
	Group A	Group B	Group C
14	2.49	3.00	3.63
16	1.64	3.01	3.31
62	1.90, 2.18	3.25, 3.58	3.24, 3.86
64			Group C off H.T. gum arabic
68			2.34, 2.38
72			1.67, 2.42
76	1.61	3.34	
77		antibiotic treatment starts	
78		4.08	
79		3.96	2.34, 2.36
82		5.68	
83		6.97	
84		6.26	
85		5.94	
86	1.87	4.11	1.91, 2.45

TABLE III.7: Volatile fatty acid analysis of rats fed a control diet (group A) plus 16% gum arabic (group B) or 16% heat-treated gum arabic (group C)

	wet weight(g) of caecal contents	dry weight(g) of caecal contents	VFA profile mg/g dry weight of caecal contents						
			A	P	iB	B	iV	V	Total
Group A	6.33	1.4	17.9	6.1	-	8.4	0.12	0.41	32.9
Group B	8.71	2.1	17.4	6.2	-	9.1	-	0.3	33.0
Group C	8.06	1.55	14.1	5.4	-	10.4	0.08	0.3	30.3

Notes:-

A - acetate

P - propionate

iB - iso-butyrate

B - butyrate

iV - iso-valerate

V - valerate

out (Table III.7) by the Wolfson Laboratory, Western General Hospital, Group B rats were given antibiotics in solution in their drinking water at a concentration of 1 $mg.ml^{-1}$. Caecal size (Table III.6) and contents were monitored on a daily basis for % dry matter and sugars present. The antibiotics used were Bacitracin, Neomycin Sulphate and Streptomycin obtained from the pharmacy of the Western General Hospital. These antibiotics in admixture have been shown (51) to have an effect on microbial behaviour in the caecum. This experiment was repeated using fourteen rats on 15% gum arabic, in order to verify the results in response to the antibiotic treatment (Table III.8).

In a separate experiment bacterial counts and volatile fatty acid analyses (Tables III.9,10) were carried out by the Medical Pathology Research Laboratory, University of Edinburgh, on the caecal contents of five rats. Rat 1 had been fed on the standard diet and rats 2-5 on the standard diet plus 15% gum arabic. Rats 3, 4 and 5 were also given the following antibiotics at a concentration of 1 $mg.ml^{-1}$ in their drinking water:- Rat 3 - Bacitracin, Neomycin Sulphate and Streptomycin; Rat 4 - Bacitracin, Neomycin Sulphate, Streptomycin and Metronidazole; Rat 5 - Metronidazole only. The rats were killed seven days after starting the antibiotic treatment and the caeca tied off and placed in an anaerobic cabinet for bacterial counts. V.F.A. analyses and % dry matter determinations were also carried out. The Metronidazole was obtained from the Veterinary College, University of Edinburgh.

TABLE III.8: Caecal weight (g) per 100 g body weight for rats fed a control diet plus 15% gum arabic and receiving antibiotics (Bacitracin, Neomycin Sulphate and Streptomycin)

Day No.	Caecal Wt(g)/100g Body weight
6	2.44, 2.74
	antibiotic treatment started
7	3.03
8	4.36
9	4.88
10	4.48
12	6.35
13	3.91
14	5.93
15	4.98
16	6.79
17	8.58
20	7.88
21	7.54

TABLE III.9: Bacterial counts for caecal contents of rats fed a control diet (rat 1) plus 15% gum arabic (rat 2) plus Bacitracin, Neomycin Sulphate, Streptomycin (rat 3) and Metronidazole (rat 4) or only plus gum arabic and Metronidazole (rat 5)

Rat No.	Approximate microscopic total bacterial count	Viable Counts					
		Aerobic			Anaerobic		
		Coliforms	G.P.C.	G.P.B.(1)	G.N.B.(2)	G.P.B.(1)	Total
1	$6x10^{10}$	$2.58x10^{7}$	$5x10^{6}$	$6.35x10^{8}$	$6.5\ x10^{8}$	$3.93x10^{9}$	$4.58x10^{9}$
2	$8x10^{10}$	$1\ x10^{5}$	$\sim 5x10^{7}$	$7.7\ x10^{8}$	$1.63x10^{9}$	$7.5\ x10^{9}$	$9.13x10^{9}$
3	$2x10^{10}$	$>1.5x10^{9}$	$<5x10^{3}$	$<5\ x10^{3}$	$2.5\ x10^{9}$	$<5\ x10^{3}$	$2.5\ x10^{9}$
4	$2.5x10^{10}$	$\sim 9.5x10^{8}$	$<5x10^{3}$	$<5\ x10^{3}$	$2.1\ x10^{9}$	$<5\ x10^{3}$	$2.1\ x10^{9}$
5	$8x10^{10}$	$3.5\ x10^{6}$	$\sim 1.5x10^{7}$	$\sim 1\ x10^{9}$	$2.23x10^{9}$	$7.77x10^{9}$	$1\ x10^{10}$

N.B.: All counts expressed as bacteria per gram wet weight of caecal contents

G.P.B.(1) - Lactobacillus-like

G.N.B.(2) - Coliforms, Bacteroides and Fusobacteria

G.P.C. - Gram positive cocci

G.P.B. - Gram positive bacilli

G.N.B. - Gram negative bacilli

> = greater than

< = less than

$\sim$ = approximate count

TABLE III.10: Volatile fatty acid analysis for caecal contents of rats fed a control diet (rat 1) plus 15% gum arabic (rat 2) plus Bacitracin, Neomycin Sulphate, Streptomycin (rat 3) and Metronidazole (rat 4) or only plus gum arabic and Metronidazole (rat 5)

Rat No.	% dry weight of caecal contents	Wet weight (g) of content used for VFA profile	V.F.A. profile µmoles/gm wet weight of caecal contents						
			A	P	iB	B	iV	V	Total
1	19.6	0.151	54.3	13.9	-	20.5	-	0.5	89.2
2	18.1	0.224	24.5	7.1	0.3	15.1	-	0.5	47.5
3	15.6	0.246	9.3	-	-	-	-	-	9.3
4	23.6	0.511	3.1	-	-	-	-	-	3.1
5	17.9	0.185	79.4	19.4	-	19.4	-	1.4	119.6

Notes:- A, P. iB, B, iV, V as in Table III.7.

III.3(iii) RESULTS AND DISCUSSION

This experimental work was completed in 1984, since when there have been many publications relevant to, and enlarging upon certain areas of this study. These findings will be incorporated in this discussion and, where possible, comparisons made between the results found.

Results from these preliminary experiments on small numbers of laboratory rats give a good indication of how rapidly caecal enlargement is induced. Table III.5 shows that, while there is no difference between control and test caecal weights after 48 hours on a 15% gum arabic (new) supplement, a very marked increase in caecal weight (grams) per 100 g body weight occurred within 48-72 hours. After the rats were no longer receiving the gum arabic supplement, a marked decrease in caecal size occurred, again within 48-72 hours (Table III.5). As the dietary transit time for a rat has been shown to be between 6 and 7 hours (28), this timing for caecal enlargement and reduction to occur, indicates a delayed reaction to the initial passage of gum arabic and, therefore, an adaptive response. Recently (52), it has been reported that a 4-day adaptation period seems adequate when evaluating the fermentability of dietary fibre in rats. In keeping with this study (52), no significant change in response was found with prolonged duration of feeding i.e. no progressive increase in caecal size with time was found in this experiment (Table III.3,5,6).

To study the effect of a slight modification of the gum, such as may occur in normal food processing, the effect of heat-treated gum arabic on caecal enlargement was looked

at. This was found to cause a similar degree of enlargement to 'normal' gum arabic and the enlargement was also found to be largely reversible.

This increase in caecal weight would appear to be solely due to an increase in the weight of caecal contents, as weights obtained for air-dried caecal tissue in these experiments remained fairly constant for test and control rats when compared as weight in grams per 100 g body weight. This is in contrast to findings by Mallet et al. (53) who found significant increases in the weight of the caecal wall as well as in the caecal contents of male rats fed a diet containing 50 g/kg of gum arabic fed ad lib. The increase in the weight of caecal contents could result from either; an increase in bacterial mass (22), the retention of water in the caecal contents, or an increase in the protein, volatile fatty acid or polysaccharide and free sugar concentration in the caecum, or a combination of these factors.

% Dry matter determinations on control and test caecal contents indicated an increase in the average % moisture content of the caecum of 80% in control animals to 84% in animals receiving 15% gum arabic. These measurements indicate that 80-90% of the increase in caecal weight of the test animals is due to an increase in the water content. However, the method of measurement used does not differentiate between free water and intracellular bacterial water.

Paper chromatography of caecal supernatant solutions and of small scale hydrolyses of these solutions were carried out on Whatman No. 1 papers with the following solvent system (v/v): benzene, butan-1-ol, pyridine, water (1:5:3:3, upper

layer). This chromatography revealed that no free sugars or soluble polysaccharide fragments attributable to gum arabic or gum arabic degradation were present. Similar analysis of the insoluble component of the caecal contents of test and control animals also failed to detect any remnants of gum arabic.

The % nitrogen content of a control caecal supernatant sample, freeze-dried, was found to be 3.87%, while that of a similar test sample was 4.49%N. If all this nitrogen arises from protein, this would give a hypothetical % protein content of the soluble caecal contents of 24% and 28% respectively. However, when amino acid analysis of these samples was carried out, their profiles were found to be almost identical (Table III.4). No correlation could be made between the amino acid profile for the test caecal sample and that for gum arabic (new), particularly obvious is the lack of any significant enrichment of hydroxyproline, serine, threonine, histidine or leucine, the five main amino acids in gum arabic (new), in the test caecal sample (Table III.2). This would tend to indicate that no amino acids attributable to gum arabic are present in the caecum.

Analysis of the microfloral content of the caeca of two control animals and four test animals showed no conclusive increase in the overall bacterial mass, but seemed to indicate a reduction in the presence of coliform organisms in the test animals. This finding has been corroborated by Mallet et al. (53) who found that, despite the increase in caecal weight, the size of the bacterial population was not significantly increased on feeding ad lib. a purified diet plus 50 g/kg gum

arabic. However they found (53) that gum arabic, guar gum and locust bean gum increased the activity of many of the bacterial enzymes. It has been stated (54) that it is unlikely that dietary fibre would have a major effect on the size of the bacterial population, but that an undetectable change in numbers might be of physiological significance. The relative insensitivity of taxonomic (bacteriological) methods may preclude the effective characterisation of diet-related changes in microbial species composition and bacterial concentration in the hind-gut (55). The enzyme activity of the gut bacteria is thought to present a more easily characterised alternative, and one of direct relevance to the toxicologist (56).

It has been suggested (57,58) that the size of the caecum is controlled by the osmotic value of its contents and should be considered an adaptive, homeostatic mechanism. In keeping with other findings (58), no significant difference in osmolality between test and control caecal contents was found in this experiment. This is to be expected as, if adaptation is complete, osmolality is maintained within normal limits. It is thought that a direct consequence of bacterial action in the caecum is the formation of short-chain (or volatile) fatty acids (59), and it is their presence that is responsible for raising the osmotic value of the caecal contents. Volatile fatty acid analysis in this experiment (Table III.7) showed no significant differences in concentrations between control and test rat caecal contents. This is in agreement with recent studies by Topping et al. (60). Previous studies in their laboratory reported (61) higher levels of volatile

fatty acid concentrations in the caecum of rats fed a gum arabic supplement, than those fed with a cellulose supplement. These differences were observed on feeding gum arabic or cellulose at 10% by weight, but their current data (60) show total V.F.A. concentrations to be the same with both preparations fed at 14% of the diet. This higher dietary concentration led to the mass of caecal V.F.A. being greatly increased with gum arabic, due to increased weight of caecal contents, a result in agreement with those found in this experiment.

In the initial experiments involving the addition of the antibiotics Bacitracin, Neomycin Sulphate and Streptomycin at 1 $mgml^{-1}$ in the drinking water of rats receiving a 15-16% supplement of gum arabic in their diet, a marked increase in caecal enlargement was evident (Tables III.6 and 8). This increased caecal enlargement was evident within 24-48 hours of the start of the treatment and appeared to be progressive with a longer duration of treatment (Table III.8). The caecal contents of rats receiving the antibiotic treatment appeared much more fluid and pockets of gas were evident. Analysis of the caecal contents showed an increase in the % dry matter present with an average 22% dry matter in the caecal contents of rats receiving antibiotics, as compared to previous values of 20% for control animals and 16% for rats receiving only the supplement of 15% gum arabic. Paper chromatography showed no distinct free sugars present in either the soluble or insoluble components of caecal contents of rats receiving antibiotics. However, after hydrolysis with 1N H_2SO_4, paper chromatography revealed a change in the sugars present in both the soluble and insoluble fractions, as compared to control

rats or rats receiving 15% gum arabic. Enrichment of rhamnose and galactose was especially apparent, suggesting the presence of gum arabic remnants in the caecum. It was thought that these differences must reflect a change in the bacterial flora of the caecum, with a loss of some of the organisms responsible for the breakdown of gum arabic. A further study was carried out on five rats, one on a control diet (Rat 1) and the remaining four receiving a 15% gum arabic supplement with:- Rat 2 - no antibiotic treatment; Rat 3 - Bacitracin, Neomycin Sulphate and Streptomycin; Rat 4 - Bacitracin, Neomycin Sulphate, Streptomycin and Metronidazole; Rat 5 - Metronidazole only, all at 1 $mgml^{-1}$ in the drinking water. Metronidazole is used to treat many protozoal and anaerobic bacterial infections. It has been reported to be metabolised by the microflora of the caecum (62). Results of bacterial counts (Table III.9) indicate that the feeding of gum arabic does not significantly affect the number (only a possible slight increase) of anaerobic bacteria which make up more than 90% of the caecal bacterial population. There may be a slight decrease in the number of aerobic gram negative bacilli accompanied by a slight increase in the aerobic gram positive cocci. The addition of Metronidazole appears to have had no discernible effect. The treatment with Bacitracin, Streptomycin and Neomycin Sulphate eliminated the aerobic gram positive cocci and bacilli and the anaerobic gram positive bacilli. Since remnants of gum arabic have been found in the caeca of rats receiving this treatment, it may be assumed that one or more of these groups is involved in gum catabolism. As this is also associated with increased caecal enlargement, as compared

to that with gum arabic alone, it can only be assumed that the osmotic effect of the gum is greater if metabolism is prevented. This would imply that the products of gum arabic metabolism are rapidly removed from the caecum or utilised, as would be expected of volatile fatty acids. V.F.A. analysis (Table III.10) showed the virtual disappearance of V.F.A.'s from the caecum of these rats with only small amounts of acetate remaining. This again indicates the loss of bacterial metabolism of gum arabic in the caecum due to the antibiotic treatment.

Recent studies have reported (63) a rapid adaptation of faecal flora on the addition of gum arabic to the diet of a human volunteer, such that the proportion of bacteria able to ferment gum arabic increased markedly. These organisms were not identified. After gum arabic was withdrawn from the diet the proportion of gum arabic fermenters returned to the level present before ingestion, confirming that the response was due to dietary change. The total viable count per gram dry weight of faeces present during the treatment period was unchanged, in contrast with the effect seen with guar gum (64), where the total count per gram dry weight of faeces increased seventeen fold but the proportion of guar fermenters stayed the same. It is suggested (64) that when guar forms part of the diet, carbohydrate, which would normally be digested in the small intestine, may pass into the colon and might thus allow greater growth of organisms other than guar gum fermenters (63). The major end products of hind-gut fermentation are short-chain fatty acids which are rapidly absorbed from the human colon (65,66) and therefore contribute to the hosts energy metabolism (67).

III.3(iv) CONCLUSIONS

1. Gum arabic causes caecal enlargement in the rat. This enlargement is not detectable in less than 48 hours, but is well established by 72 hours. Caecal enlargement is reversible and the reversibility is well established within 72 hours of the cessation of feeding gum arabic. The weight of the washed and isolated tissue that constitutes the caecum does not increase significantly. The increase in weight of the caecum is therefore due to an increase in weight of caecal contents. Small differences in caecal size may reflect an adaptive change to differences in the diet and it is probably only justifiable to attach toxicological significance to results when gross effects, accompanied by secondary nutritional and toxicological changes are observed, as in the case of raw potato starch (58).

2. No significant differences were found in the osmotic pressure or total bacterial, volatile fatty acid, % sugar or amino acid concentrations in the caecal contents of rats fed a 15% gum arabic supplement. However a 4% increase in moisture content was evident.

3. The heat treatment of gum arabic did not appear to change its effects on caecal enlargement in rats.

4. Treatment of rats receiving 15% gum arabic with the antibiotic Metronidazole appeared to have no discernible effect. However, the antibiotic cocktail Bacitracin, Neomycin Sulphate and Streptomycin, greatly affected the digestion of gum arabic in the rat. Caecal enlargement was greatly

increased, an increase which was evident within 24-48 hours and appeared to be progressive with the duration of treatment. This increase reflected an increase in the % dry matter of the caecal contents of 6%, some of which would appear to be accounted for by remnants of gum arabic, as acid hydrolysis of caecal contents revealed an enrichment of galactose and rhamnose. Bacterial counts revealed the elimination of the aerobic gram positive cocci and bacilli and the anaerobic gram positive bacilli populations in the caecum and also an almost complete elimination of volatile fatty acid concentrations, indicating the fermentation of gum arabic, by one or more of these groups, to volatile fatty acids in the caecum has been prevented.

Although further studies along these lines would have been desirable, they were discontinued for two reasons: (a) the publication of several papers by other workers in this field attracted by the Edinburgh studies published in 1981-82, and (b) the withdrawal of bacteriological facilities because the bacteriologists considered that the data obtained thus far did not justify their further participation as a result largely of pressure upon them to start urgently on more serious problems involving matters of public health and epidemiology.

CHAPTER III.4. DEGRADATION OF GUM ARABIC BY MILD ACID HYDROLYSIS, AUTOHYDROLYSIS, AND ULTRA-VIOLET IRRADIATION

III.4(i) INTRODUCTION

It has long been established that Acacia exudates are based on highly branched galactan frameworks carrying arabinose side-chains (i.e. are arabinogalactans) (68) and that variable amounts of proteinaceous (or polypeptide) material are also present (13,69). They can therefore be regarded as arabinogalactan-proteins; such complex polymer structures are becoming widely recognised from a variety of phytochemical sources, as reviewed by Professor Stone and his co-workers (18,70).

Arabinogalactan-proteins are a group of macromolecules characterised by a high proportion of carbohydrate (typically 80-95%) in which galactose and arabinose are the predominant monosaccharides; there is also a low proportion of protein, typically containing high levels of hydroxyproline (18). Arabinogalactan-proteins are found in most higher plants and in many of their secretions. Yariv (71) showed that artificial carbohydrate antigens would precipitate gum arabic and polysaccharides containing arabinose and galactose from soya bean, jack bean and maize flour. This finding has been extended by Jermyn and Yeow (72) to the isolation of high molecular weight polymers, containing carbohydrate and protein in the ratio approximately 8:1, from seeds representing all taxonomic groups of higher plants, as well

as from other plant tissues (73). Although arabinogalactan-proteins are widely distributed in the plant kingdom no general function has been ascribed to them and it can only be speculated that they play some fundamental role or fulfil different functions in different situations (18,70).

The physical properties of arabinogalactan-proteins depend on the structures of both the polypeptide and the substituent arabinogalactan polysaccharides. The lack of precise information regarding the carbohydrate-protein linkage and organisation of these molecules makes it very difficult to predict their physical behaviour (18). The glycosylated protein component is resistant to proteolysis (74), presumably because of its substitution with bulky carbohydrate groups. Many attempts have been made to identify the point of attachment of the arabinogalactan to the protein portion of the molecule and differing results have been obtained. In wheat endosperm, an arabinogalactan-peptide has been isolated in which hydroxyproline is linked in an alkali-stable glycosidic linkage to the β-D-galactopyranosyl residue of the reducing terminus of the arabinogalactan (75,76) while in rice bran proteoglycan (77) an α-arabino-furanosyl residue is linked to hydroxyproline. In Cannabis sativa leaves (74), both a galactopyranosyl-serine linkage and an alkali resistant carbohydrate-protein linkage are present, while in Phaseolus vulgaris arabinogalactan-protein, carbohydrate is attached to serine, threonine and hydroxy-proline (78). Studies by Akiyama et al. (8) on gum arabic suggest the presence of an hydroxyproline-arabinose linkage and also a serine-carbohydrate linkage. Recent reports on

the exudate from Acacia erioloba (syn. giraffe, Bentham Series 4, Gummiferae) indicate that little, if any, of its carbohydrate is present as free polysaccharide. The carbohydrate is distributed in the glycoprotein in the form of substituents on hydroxyproline which have various short chain-lengths; the hydroxyproline residues are in peptide chains. Both arabinose-hydroxyproline and galactose-hydroxyproline linkages were identified (79,80).

The presence of hydroxyproline in both soluble and insoluble plant proteoglycans and glycoproteins is now well established (18,70) although for many years it was considered solely as a constituent of animal proteins. It is generally accepted that peptidyl hydroxyproline arises from a post-translational modification of proline and not from incorporation of free hydroxyproline (81).

The identification of gum arabic (Acacia senegal) as a proteoglycan may explain its hitherto poorly understood behaviour in certain food manufacturing processes. Natural gum arabic carries a wide range of yeasts, fungi, moulds (including Aspergillus spp.) and other microorganisms including thermophilic spore-forming bacilli (B. stearothermophilus) of remarkable tolerance to heat treatment. Thermal or other sterilisation processes are necessary to ensure that gum arabic conforms to the established microbiological criteria for pharmaceutical use (82); a microbiological specification for food additive use is under consideration by J.E.C.F.A. Heat may be generated when natural gum is milled to a fine powder; heating is involved in the inactiva-

tion of the enzymes naturally present or when batches of gum are dissolved as quickly as possible in the preparation of the modern spray-dried product. There are some data available for the lability of the peripheral monosaccharides in gum arabic (83,84), gum tahla (14) and other Acacia exudates (85-89) during the heating of gum solutions (i.e. autohydrolysis; Acacia gums give solutions of pH 4.2-4.8) and during mild acid hydrolysis. The thermal effect on the other gum components has not, however, been studied.

Earlier studies recorded that Acacia and other exudates (14,83-90) were inexplicably sensitive to autohydrolysis and that flocculent precipitates, shown to be proteinaceous (84,86) were formed. The identity of the amino acids involved in these precipitates was not investigated. Following a study of the amino acid composition of several gum arabic samples (13), studies of the sugar/amino acid relationships in the sequential Smith-degradation products obtained from the exudates from A. polyacantha (91) and A. senegal (Chapter III.5 of this thesis) have indicated that some amino acids are involved in peripheral positions in the gum macromolecules, with other amino acids located more extensively at the branched galactan core of the highly branched framework. The isolation (84,92) of a high molecular weight fraction of high nitrogen content and of a low molecular weight fraction having no nitrogen present implies that gum arabic contains polysaccharide sub-units interspersed with peptide linking units. It was therefore important to gain a more extensive knowledge of the identity and proportions of those amino acids located at or near the periphery of

undegraded gum molecules in order that a greater understanding of the true structure of gum arabic, of the allergenicity attributable to it (38,40), and of its unique functionality as an emulsifier for citrus flavouring oils, may be derived; heating and treatment with proteases reduce the emulsification efficiency (93).

This study presents the data obtained from a study of the fate of the sugars and amino acids in gum arabic solutions subjected to autohydrolysis, mild acidic hydrolysis, and ultraviolet irradiation.

III.4(ii) METHODS AND MATERIALS

A spray-dried commercial sample of gum arabic (designated "gum arabic new") was used in all experiments.

Autohydrolysis - Autohydrolysis was carried out by heating 28.5 g gum, as a 2% (w/v) aqueous solution, pH 4.6, under reflux on a boiling water bath for 48 hours. A brown, flocculent precipitate formed. The solution was cooled and centrifuged (Sorvall RC-SB refrigerated centrifuge) at 10,000 r.p.m. for 35 minutes. The insoluble centrifugate was washed, suspended in water, and freeze-dried (yield 0.05 g). The residual solution was dialysed against distilled water for 24 hours; the dialysate was concentrated (rotary evaporator) at 38°C then freeze-dried (yield 1.4 g). The contents of the dialysis bag were dialysed for a further 48 hours against running tap water before being concentrated (rotary evaporator) and freeze-dried (yield 23.4 g).

In an earlier study of the autohydrolysis of gum arabic solutions (84), electrodialysed gum (i.e. arabic acid) having a pH of 2.8 was used. In this experiment, a solution of the gum in its natural form was used (pH 4.8) as this applies more directly to manufacturing practice and also results in less drastic degradation.

Mild acidic hydrolysis - Gum arabic (3.8 g) was dissolved in 200 ml 0.005 M sulphuric acid and hydrolysed on a boiling water bath for 48 hours. After cooling, the solution was fractionated as described under autohydrolysis above.

Yields:- 0.004 g insoluble precipitate; 1.51 g diffusate; 1.78 g degraded gum.

Ultraviolet irradiation - Gum arabic, 23.8 g in 200 ml distilled water, was irradiated (Hanovia Model 11 ultraviolet lamp, 125 watt) for 16 hours. The solution was stirred gently during the irradiation. An insoluble precipitate formed and the solution temperature rose slowly to 55°C over the 16 hours. (When a similar gum solution was heated slowly to 55°C over 16 hours, without ultraviolet irradiation, no precipitate formed.) The irradiated solution was fractionated as described under autohydrolysis above.

Yields:- 0.36 g insoluble precipitate; 0.41 g diffusate; 20.0 g degraded gum. The total recovery was therefore 87% in all three degradative experiments.

III.4(iii) RESULTS

The data obtained for the sugar compositions etc. of the gum arabic and the residual gums obtained from each of

the three degradation processes are shown in Table III.11. Table III.12 shows the corresponding data for the insoluble precipitates and diffusates from the three degradation processes. Tables III.13, 14 and 15 show the amino acid data for the three products obtained by autohydrolysis, mild acidic hydrolysis, and ultraviolet irradiation respectively. Table III.16 shows the recoveries of the various degradation products and the percentages of the sugars and the amino acids, originally present in gum arabic, that are located in each degradation product (as distinct from the sugar and amino acid compositions of these products).

III.4(iv) DISCUSSION

The data in Tables III.11 and 12 indicate that autohydrolysis and ultraviolet irradiaton, under the conditions described, lead to degradations that are very similar in extent, as revealed by the loss in viscosity (from 15 mlg^{-1} to 10 mlg^{-1}, Table III.11) and recoveries (82 and 84% respectively) of the degraded gums. Mild acidic hydrolysis led to more drastic degradation, as indicated by the decreased recovery (47%) having a much smaller viscosity (6 mlg^{-1}): the degraded gum was also much more depleted in arabinose and rhamnose. The sugars eliminated from the original gum molecules appear in either the insoluble or diffusate fractions; calculation shows that the sugar recoveries are approximately quantitative when corrected for the total overall yields of 87% obtained for each method of degradation used. Earlier studies revealed that, in acidic degradations, the diffusate contains free galactose, arabinose, rhamnose,

TABLE III.11 Analytical data for gum arabic and the degraded products formed after autohydrolysis, mild acid hydrolysis, or ultra-violet irradiation

	Gum Arabic	Degraded products after autohydrolysis	Degraded products after mild acid hydrolysis	Degraded products after UV irradn.
Recovery, %	-	82	47	84
Moisture, %	5.0	4.1	13.5	7.9
Ash, %[a]	3.6	3.8	3.8	3.2
Nitrogen, %[a]	0.34	0.26	0.50	0.22
Nitrogen conversion factor (N.C.F.)[b]	6.43	6.66	6.75	6.93
Hence protein, % (NxN.C.F.)[a]	2.19	1.73	3.38	1.52
Specific rotation in water (degrees)[a]	-20	-19	-8	-20
Intrinsic viscosity, mlg^{-1} [a]	15	10	6	10
Equivalent weight[a]	980	980	610	1130
Hence uronic anhydride, %	18	18	29	16
Sugar composition after hydrolysis, %[c]				
Glucuronic acid[d]	18	18	29	16
Galactose	48	50	59	50
Arabinose	25	24	10	25
Rhamnose	9	8	2	9

Notes:

[a] Corrected for moisture content.

[b] From Tables III.13, 14 and 15.

[c] Corrected for protein content.

[d] Including 4-O-methylglucuronic acid.

TABLE III.12 Analytical data for gum arabic, the insoluble material and diffusate formed after auto-hydrolysis, mild acid hydrolysis, and ultra-violet irradiation

	Gum arabic	Auto-hydrolysis		Mild Acid hydrolysis		U.V. Irradiation	
		insoluble material	diffusate	insoluble material	diffusate	insoluble material	diffusate
Recovery, %	-	0.2	5.0	0.1	40	1.5	1.7
Moisture, %	5.0	4.5	4.8	6.2	8.6	7.4	7.0
Ash, %[a]	3.6	n.d.	6.3	n.d.	7.2	n.d.	n.d.
Nitrogen, %[a]	0.34	11.10	0.20	10.0	0.10	2.87	1.97
Nitrogen conversion factor[b]	6.43	6.48	7.02	6.71	6.11	6.54	6.90
Hence protein, % (NxN.C.F.)	2.19	71.9	1.4	67.1	0.6	18.8	13.6
Sugar composition after hydrolysis, %[c]							
Glucuronic acid[d]	18	n.d.	0	n.d.	5	n.d.	2
Galactose	48	46	25	45	36	52	20
Arabinose	25	44	55	38	42	36	58
Rhamnose	9	10	20	16	17	12	20

Notes: n.d. = not done

a,b,c,d As in Table III.11

TABLE III.13 Amino acid compositions of gum arabic and the products recovered after autohydrolysis

	Gum arabic	degraded gum	insoluble material	diffusate
% Nitrogen	0.34	0.26	11.1	0.20
Alanine	32	23	83	0
Arginine	6	2	13	0
Aspartic acid	65	34	118	268
Cystine	1	0	0	2
Glutamic acid	35	27	97	76
Glycine	44	38	92	103
Histidine	73	50	47	27
Hydroxyproline	262	289	7	77
Isoleucine	14	7	42	22
Leucine	72	78	103	48
Lysine	22	16	23	4
Methionine	1	0	2	4
Phenylalanine	27	18	64	36
Proline	71	82	52	96
Serine	143	186	79	128
Threonine	77	105	46	41
Tyrosine	11	11	16	10
Valine	47	31	117	59
Hence Nitrogen Conversion Factor	6.43	6.66	6.48	7.02

TABLE III.14 Amino acid compositions of gum arabic and the products recovered after mild acid hydrolysis

	Gum arabic	degraded gum	insoluble material	diffusate
% Nitrogen	0.34	0.50	10.0	0.10
Alanine	32	0	94	67
Arginine	6	0	0	0
Aspartic acid	65	35	89	128
Cystine	1	0	0	0
Glutamic acid	35	21	92	76
Glycine	44	40	79	73
Histidine	73	52	47	107
Hydroxyproline	262	376	22	83
Isoleucine	14	7	47	24
Leucine	72	77	116	66
Lysine	22	7	11	16
Methionine	1	0	0	0
Phenylalanine	27	13	63	37
Proline	71	72	55	52
Serine	143	157	88	133
Threonine	77	106	45	59
Tyrosine	11	10	17	10
Valine	47	27	135	69
Hence Nitrogen Conversion Factor	6.43	6.75	6.71	6.11

TABLE III.15 Amino acid compositions of gum arabic and the products recovered after ultra-violet irradiation

	gum arabic	degraded gum	insoluble material	diffusate
% Nitrogen	0.34	0.22	2.87	1.97
Alanine	32	10	83	67
Arginine	6	2	21	3
Aspartic acid	65	50	133	144
Cystine	1	0	0	0
Glutamic acid	35	25	90	67
Glycine	44	37	100	112
Histidine	73	71	16	0
Hydroxyproline	262	309	20	143
Isoleucine	14	11	43	16
Leucine	72	62	109	81
Lysine	22	23	28	0
Methionine	1	0	0	0
Phenylalanine	27	17	47	15
Proline	71	71	43	98
Serine	143	177	95	151
Threonine	77	78	43	45
Tyrosine	11	12	11	0
Valine	47	45	118	58
Hence Nitrogen Conversion Factor	6.43	6.93	6.54	6.90

TABLE III.16 Percentage of original amino acids and sugars in gum arabic appearing in each fraction after degradative processes

	Degraded gum			Insoluble fraction			Dialysate		
	% weight	Amino acids	Sugars	% weight	Amino acids	Sugars	% weight	Amino acids	Sugars
Autohydrolysis	82	87%	97% Gal 88% Ara 87% Rha	0.2	8.8%	0.2% Gal 0.4% Ara 0.3% Rha	5.0	4.2%	3% Gal 12% Ara 13% Rha
Mild acidic hydrolysis	47	84%	62% Gal 22% Ara 1.5% Rha	0.1	3.5%	<0.1% Gal <0.1% Ara <0.1% Rha	40	12.5%	38% Gal 78% Ara 98% Rha
Ultraviolet irradiation	84	71.5%	98% Gal 94% Ara 94% Rha	1.5	15.5%	1% Gal 2% Ara 2% Rha	1.7	13%	1% Gal 4% Ara 4% Rha

and galactose- and arabinose-containing di- and tri-saccharides, together with smaller amounts of acidic components (83,84).

In each degradative process, there was confirmation of the formation of the insoluble, proteinaceous material previously observed (83-89); the yield of this fraction was greatest in the ultraviolet irradiation, but its nitrogen content (2.87%) was much less than was given by the auto-hydrolysis (N = 11.1%) and mild acidic hydrolysis (N = 10.0%) procedures. In contrast, the ultraviolet diffusate had a much higher nitrogen content (1.97%) than the diffusate from the acid procedures (0.2 and 0.1% N respectively). The physical degradation produced by ultraviolet irradiation therefore appears to involve a different mechanism than the acidic degradations.

The insoluble products and diffusates from all three degradation processes contain both sugars and amino acids. Tables III.13, 14 and 15 show, for each of the degradation processes, the proportions of the amino acids originally present that remain in the degraded gums, and the proportions of the amino acids that are eliminated and hence appear in either the insoluble precipitates or the diffusates. Alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine and valine are major components of the insoluble fractions; aspartic acid, glutamic acid, glycine and serine are the major components of the diffusates. The insoluble components contain very little hydroxyproline; the degraded gums isolated therefore contain slightly increased relative proportions of hydroxyproline as well as of proline, serine and threonine. It is of interest that

enrichment of these same amino acids occurs in the peptides associated with the branched galactan core of the original gum that results from several Smith-degradations of the gums from Acacia senegal (Chapter III.5 of this thesis) and Acacia polyacantha a close botanical relative of A. senegal (91). As discussed in the introduction to this section (8, 74-78), these amino acids participate in carbohydrate-protein linkages in arabino-galactan proteins.

Analysis of the data obtained for gum arabic reveals that its sugars and amino acids differ in their relative stabilities to autohydrolysis, mild acidic and ultraviolet degradation (Table III.16). On mild acidic hydrolysis, the degraded gum retains 84% of the original protein but only relatively small amounts of the original galactose and arabinose; almost all of the original rhamnose appears in the dialysate, together with 12.5% of the original protein, and with 38% and 78% respectively, of the original galactose and arabinose. The protein content of gum arabic is even more sensitive to ultraviolet degradation, with 28.5% of the original protein being eliminated; it appears with minor amounts of the original sugars, in the insoluble fraction (15.5%) and dialysate (13%) respectively. Autohydrolysis appears to affect the protein content and sugar contents of gum arabic to similar extents, with 87% of the original protein and 97% of the galactose, 88% of the arabinose and 87% of the rhamnose remaining. However of the 13% protein eliminated, 8.8% appears in the insoluble fraction with only traces of sugars, while the dialysate fraction contains most of the sugars eliminated and only 4.2% of the protein.

Heat treatments of gum arabic solutions, e.g. in food processing, must therefore be minimised if degradative changes and protein denaturation are to be avoided. An assessment of the lability of some of the amino acids in gum arabic when its solutions are heated, and of the extent of the formation of insoluble material containing sugars and amino acids, has therefore been obtained. That information, and awareness of the possible resistance of these materials to proteolysis, is necessary for an understanding of the source of some of the metabolic and immunological effects attributed to gum arabic and other comparable macromolecules. New insight into the ramifications and complexity of the amino acids present in gum arabic, and their contributions to its molecular structure and functionality has also been obtained.

CHAPTER III.5. SEQUENTIAL SMITH DEGRADATIONS OF GUM ARABIC (OLD)

III.5(i) INTRODUCTION

Smith and his co-workers first applied the technique of periodate oxidation, borohydride reduction, and controlled acid hydrolysis to gum arabic in 1959 (94). Following this, sequential Smith degradation was introduced by Anderson, Hirst and Stoddart (83). Each Smith degradation involves a periodate oxidation stage followed by a reduction with borohydride; the resulting polyalcohol is hydrolysed under very mild, acidic conditions in order to cleave the glycosidic linkages of only the periodate-opened sugar residues. Thus,

for example, galactose residues linked (1-6) are susceptible to this degradation but residues linked (1-3) remain intact. Smith degradation data and data from autohydrolysis and other hydrolytic degradations have been reviewed by Street and Anderson (19) and a refinement of structure proposed for gum arabic and other Acacia gum exudates. Churms et al. (6) have also proposed a structure for gum arabic using information from Smith degradations, and suggest the presence in the polysaccharide chain of uniform blocks of (1-3)-linked D-galactanopyranosyl residues.

Recently, data has been published which takes into account the fate of the proteinaceous component of three different Acacia gums; Acacia polyacantha (91); A. robusta (95); and A. tortilis (96) on sequential Smith degradation. It was found that successive degradations led to an enrichment of the protein content of the galactan core of A. polyacantha, but not of A. robusta and A. tortilis. Their nitrogen content was shown to become enriched after mild acid hydrolysis (as was the case with gum arabic (new) in Chapter III.4 of this thesis), but severely depleted on successive Smith degradations (95,96). These two Acacia species are both members of Bentham's Series 4 (Gummiferae) while A. polyacantha is a member of Bentham's Series 5 (Vulgares), and it is possible that these groups differ in the location of proteinaceous material within their complex molecular structure. A. polyacantha (formerly named Acacia campylacantha) is regarded (12) as a close botanical relative of A. senegal (gum arabic) and therefore a series of Smith degradations was carried out on gum arabic (old) in this

study, and each sequential product analysed for both its polysaccharide and proteinaceous components. More detailed information on the nature, location and biodegradability of the proteinaceous components of natural gums would clearly be an advantage in nutritional, medical and toxicological studies as well as in studies of the true molecular structure of the Acacia gums.

III.5(ii) SEQUENTIAL SMITH DEGRADATIONS

Gum arabic (old), (20 g) was dissolved in distilled water (500 ml) and 0.25 M-sodium metaperiodate solution (500 ml) was added. The oxidation (in darkness, while stirring at room temperature) was followed by measuring the release of formic acid with time. After 48 hours, the reaction was stopped by the addition of ethylene glycol (10 ml): the solution was dialysed against running tap water for 2 days. Sodium borohydride (5 g) was added and the mixture kept at room temperature for 30 hours, then dialysed for a further 2 days. The polyalcohol was hydrolysed in N-sulphuric acid at room temperature for 2 days after which the solution was neutralised (barium carbonate), filtered, deionised (Amberlite resin IR-120(H)), reduced in volume to ca. 250 ml, and dialysed against distilled water (1 l). After dialysis against running tap water for 2 days, fraction SDI was isolated as the freeze-dried product. As the yield was found to be low (5.023 g, 25% yield), this stage was repeated twice (4.993 g and 5.268 g, 25% and 26% yield, respectively) and the products pooled to give SDI

(% N = 0.56; $[\alpha]_D$ = -17.3; $[\eta]$ = 9.5; glucuronic acid = 4%, galactose, 73%; arabinose, 22%; rhamnose, 1%).

Borohydride reduction of periodate-oxidised SDI (10 g), followed by controlled acid hydrolysis at room temperature with N-sulphuric acid for 2 days, gave SDII (5.130 g; 51% yield; % N = 0.87; $[\alpha]_D$ = +13.0; $[\eta]$ = 11.0; galactose, 97%; arabinose, 3%). (The N-sulphuric acid solution was not neutralised with barium carbonate in this case, nor in subsequent Smith degradations; degraded products were isolated after dialysis of the acidic solutions against running tap-water for 2 days. This procedure was adopted because polysaccharide material, which often precipitated from acidic solutions, redissolved during dialysis.)

Borohydride reduction of periodate oxidised SDII (4.0 g), followed by controlled acid hydrolysis with N-sulphuric acid for 2 days at room temperature gave SDIII (2.479 g; 62% yield; % N = 0.90; $[\alpha]_D$ = +20.0; galactose, 97%; arabinose, 3%).

Borohydride reduction of periodate oxidised SDIII (1.5 g), followed by controlled acid hydrolysis with N-sulphuric acid for 2 days at room temperature gave SDIV (1.191 g; 79% yield; % N = 0.85; $[\alpha]_D$ = +21.0; galactose, 100%).

III.5(iii) RESULTS

The yields and analytical data obtained for the sugar compositions of gum arabic and its sequential Smith degradation products SDI-SDIV are given in Tables III.17 and 18. The yields shown are corrected for the small aliquots

withdrawn to monitor the progress of the degradation sequences, and for the amounts used analytically to characterise each product. When the yield of SDI from 20 g of gum arabic (old) was found to be lower than that (69%) reported previously (83) for a different sample of Acacia senegal (not a commercial sample) the first Smith degradation stage was repeated a further twice on portions of gum arabic (old). The products SDI were pooled; 25% was the average recovery from the three separate preparations (25%, 25% and 26% respectively) of SDI. Table III.19 shows the results (mol/1000 mol of the proteinaceous component) obtained from amino acid analyses of gum arabic and its Smith-degradation products SDI-SDIV, together with the method of calculation used to derive quantitative values for the amino acids present. Table III.20 presents the values (derived as in Table III.19) for the amino acids in SDI-SDIV; the amounts (mmol) of each amino acid lost, in the four degradation stages overall, are also shown. Table III.17 includes values calculated for the molar and weight/weight ratios of the polysaccharide/protein present in gum arabic and its degradation products. Table III.18 includes the values calculated for the ratios of the sugars/amino acids eliminated at each stage of the degradation sequence. The data in Table III.19 permitted calculation of the nitrogen conversion factors (Table III.17) and hence the amounts of proteinaceous and polysaccharide components in each product.

TABLE III.17 Relative proportions of sugars and amino acids in Acacia senegal gum (gum arabic (old)) and its Smith-degradation products

	Yield (%)	Hence weight (g)	Nitrogen (%) and factor[a]	Hence composition of products				Hence ratios:- polysaccharide / protein	
				Poly-saccharide		Protein			
				(g)	(mM)	(g)	(mM)	g/g	mM/mM
Whole gum	-	40	0.34 (x6.76)	39.1	226[b]	0.92	7.36[c]	$\frac{39.1}{0.91}=\frac{42}{1}$	$\frac{226}{7.36}=\frac{31}{1}$
SD I	25% of 40g	10	0.56 (x6.79)	9.62	56	0.38	3.07[c]	$\frac{9.62}{0.38}=\frac{25}{1}$	$\frac{56}{3.07}=\frac{18}{1}$
SD II	51.3% of 10g	5.13	0.87 (x6.80)	4.83	27	0.30	2.41[c]	$\frac{4.83}{0.30}=\frac{16}{1}$	$\frac{27}{2.41}=\frac{11}{1}$
SD III	62% of 5.13g	3.18	0.90 (x6.61)	2.99	16.6	0.19	1.57[c]	$\frac{2.99}{0.19}=\frac{16}{1}$	$\frac{16.6}{1.57}=\frac{11}{1}$
SD IV	79% of 3.18g	2.51	0.85 (x6.52)	2.37	13.2	0.14	1.15[c]	$\frac{2.37}{0.14}=\frac{17}{1}$	$\frac{13.2}{1.15}=\frac{11}{1}$

Notes:

[a] Factors for converting %N to % protein calculated from data in Table III.19

[b] See Table III.18

[c] See Tables III.19 and 20.

TABLE III.18 Elimination of sugars and amino acids from *Acacia senegal* gum (gum arabic (old)) in sequential Smith-degradations

	Molar ratios of sugars	Hence g per 100 mol	Hence sugars in product (g)	(mM)	Hence loss of sugars (g)	(mM)	Amino acids in product (g)[a]	(mM)[b]	Hence ratios for sugars / amino acids eliminated (g/g)	(mM/mM)
Whole gum (40g) (97.7% sugars 2.3% protein)	Gal 45	8100	18.32	102			0.92	7.36		
	Ara 24	3600	8.14	54						
	Rha 14	2296	5.19	32						
	UA[c] 17	3298	7.46	38						
	100	17284	39.11	226						
SDI (10g) (9.62 g sugars)	Gal 73	13140	7.02	39	11.30	63	0.38	3.07	(39.1-9.62)/(0.92-0.38) = 55/1	(226-56)/(7.36-3.07) = 40/1
	Ara 22	3300	2.12	14	6.02	40				
	Rha 1	164	0.10	1	5.09	31				
	UA[c] 4	776	0.38	2	7.08	36				
	100	17380	9.62	56	29.49	170				
SDII (5.13 g) (4.83 g sugars)	Gal 97	17460	4.71	26	2.31	13	0.30	2.41	(9.62-4.83)/(0.38-0.30) = 60/1	(56-27)/(3.07-2.41) = 44/1
	Ara 3	450	0.12	1	2.00	13				
	Rha 0	0	0	0	0.10	1				
	UA 0	0	0	0	0.38	2				
	100	17910	4.83	27	4.79	29				
SDIII (3.18 g) (2.99 g sugars)	Gal 97	17460	2.90	16	1.81	10	0.19	1.57	(4.83-2.99)/(0.30-0.19) = 16/1	(27-16.6)/(2.41-1.57) = 12/1
	Ara 3	450	0.09	0.6	0.03	0.4				
	100	17910	2.99	16.6	1.84	10.4				
SDIV (2.51 g) (2.37 g sugars)	Gal 100	18000	2.37	13.2	0.53	3	0.14	1.15	(2.99-2.37)/(0.19-0.14) = 12/1	(16.6-13.2)/(1.57-1.15) = 8/1
	Ara 0	0	0	0	0.09	0.6				
					0.62	3.6				

Notes: [a] See Table III.17; [b] See Tables III.19 and 20; [c] 15.5% glucuronic acid + 1.5% 4-O-methylglucuronic acid in whole gum; 4% glucuronic acid in SDI (12).

TABLE III.19 The amino acid composition of Acacia senegal gum (gum arabic (old)) and its sequential Smith-degradation products

Amino acid	Mol. wt.	Acacia senegal gum							
		Mol./ 1000 mol.[a]	∴ Relative wts. present (g)	Hence wt. per 0.92 g (g)[b]	Hence mmol/ 0.92 g	Mol. per 1000 mol in Smith-degradation products			
						SDI	SDII	SDIII	SDIV
Column	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
Ala	89	31	2.76	0.020	0.22	8	8	14	15
Arg	174	7	1.22	0.009	0.05	0	0	0	0
Asp	133	63	8.38	0.061	0.46	11	17	19	25
Cys	121	0	0	0	0	0	0	0	0
Glu	147	35	5.15	0.037	0.25	6	6	8	12
Gly	61	48	2.93	0.021	0.34	31	29	34	42
His	155	39	6.05	0.044	0.28	50	50	58	57
Hyp	131	313	41.00	0.297	2.27	454	465	335	258
Ile	131	13	4.06	0.029	0.22	0	0	0	0
Leu	131	73	9.56	0.069	0.53	65	65	82	93
Lys	146	25	3.65	0.026	0.18	0	0	0	0
Met	149	1	0.15	0.001	0.01	0	0	0	0
Phe	165	27	4.45	0.032	0.19	5	5	9	9
Pro	115	81	9.32	0.068	0.59	71	83	112	124
Ser	105	111	11.65	0.085	0.81	189	166	189	205
Thr	119	79	9.40	0.068	0.57	104	95	125	142
Tyr	181	10	1.81	0.013	0.07	0	0	0	0
Val	117	45	5.26	0.038	0.32	7	11	15	18
Totals		1001	126.80	0.918g	7.36mM	1001	999	1000	1000

Notes: [a] From (13)

[b] See Table III.17

TABLE III.20 Quantitative relationships for the amino acids in Acacia senegal gum (gum arabic (old)) and its Smith-degradation products

Amino acid	mmol in whole gum [a]	Relative proportions[b] of amino acids in SDI (g in 0.38g)	(mM)	SDII (g in 0.30g)	(mM)	SDIII (g in 0.19g)	(mM)	SDIV (g in 0.14 g)	(mM)	Amino acids eliminated[c] (mM)	(%)
Column	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
Ala	0.22	0.002	0.02	0.002	0.02	0.003	0.02	0.002	0.017	0.203	92
Arg	0.05	0	0	0	0	0	0	0	0	0.05	100
Asp	0.46	0.005	0.04	0.005	0.03	0.004	0.03	0.004	0.029	0.431	94
Cys	0	0	0	0	0	0	0	0	0	-	-
Glu	0.25	0.003	0.02	0.002	0.01	0.002	0.01	0.002	0.014	0.236	95
Gly	0.34	0.006	0.10	0.004	0.07	0.004	0.07	0.003	0.047	0.293	86
His	0.28	0.024	0.15	0.019	0.12	0.014	0.09	0.010	0.065	0.215	77
Hyp	2.27	0.185	1.41	0.148	1.12	0.068	0.52	0.039	0.296	1.974	87
Ile	0.22	0	0	0	0	0	0	0	0	0.22	100
Leu	0.53	0.026	0.20	0.021	0.16	0.016	0.13	0.014	0.107	0.423	80
Lys	0.18	0	0	0	0	0	0	0	0	0.18	100
Met	0.01	0	0	0	0	0	0	0	0	0.01	100
Phe	0.19	0.003	0.02	0.002	0.01	0.002	0.01	0.002	0.010	0.18	95
Pro	0.59	0.025	0.22	0.023	0.20	0.020	0.18	0.016	0.143	0.447	76
Ser	0.81	0.062	0.56	0.042	0.40	0.030	0.29	0.025	0.235	0.575	71
Thr	0.57	0.038	0.32	0.027	0.23	0.023	0.19	0.019	0.163	0.407	71
Tyr	0.07	0	0	0	0	0	0	0	0	0.07	100
Val	0.32	0.001	0.01	0.003	0.03	0.003	0.03	0.002	0.020	0.30	94
Totals	7.36	0.380	3.07	0.298	2.41	0.189	1.57	0.138	1.146	6.214	

Notes: [a] From Table III.19, column (e)

[b] Calculated from Table III.19, columns f-i respectively

[c] Column j = columns (a-i); column k = columns $(^{j}/_{a}) \times \frac{100}{1}$

III.5(iv) DISCUSSION

Table III.17 shows the yields of the four sequential degradation products (SDI-SDIV) and their nitrogen contents: calculations of the corresponding number of millimoles of sugars and amino acids eliminated at each stage (Table III.18) were derived from data presented in Tables III.17, 19 and 20. From these results, the molar ratio of sugars/amino acids in gum arabic (old) is 31:1 and this ratio is decreased to 18:1 (SDI) and to 11:1 (SDII-SDIV). Of the original polysaccharide (39.1 g) and protein (0.92 g) in 40 g of whole gum, only 2.37 g of degraded polysaccharide (a galactan) and 0.14 g of proteinaceous or peptide material remain in SDIV. Thus 94% of the original polysaccharide but only 84% of the protein is eliminated; the degradation products become progressively more proteinaceous than the original gum. For Acacia polyacantha (91), the molar ratio of sugars/amino acids decreased from 28:1 (whole gum) to 14:1, 14:1, 12:1 and 9:1 (products SDI-SDIV); 96.5% of the original polysaccharide but only 89% of the protein were eliminated. Thus there is a distinct similarity in the sequence of degradative changes for both gums.

Table III.18 shows the number of millimoles of sugars eliminated at each stage of the degradation sequence. The first stage eliminated 62% of the galactose, 78% of the arabinose, 97% of the rhamnose and 95% of the uronic acids present originally in the gum. Thus 76% i.e. 172 mmol of the original sugars but only 4.29 mmol of the original amino acids (Table III.18) were eliminated, a molar ratio of 40:1

(112:1 for A. polyacantha gum (91)). The peripheral periodate-vulnerable galactose, arabinose, rhamnose and uronic acid residues are therefore not associated with extensive amounts of amino acids although it would appear that gum arabic (Acacia senegal) has a greater number of peripheral amino acids than A. polyacantha.

Table III.18 shows that a similar situation occurs with the second degradation step. Degradation of SDI to give SDII eliminated 33%, 92%, 100% and 100% of its galactose, arabinose, rhamnose and uronic acids respectively, corresponding to the loss of 29 mmol. of sugars. The loss of amino acids was 0.66 mmol.; thus 44 mmol. sugars were lost per mmol. of amino acids, as in the first degradative step. This ratio is higher than that found for A. polyacantha (14:1, (91)) and thus there are fewer amino acids associated with the sugars eliminated during the second stage of periodate oxidation, i.e. those residues that are located next to sugars occupying chain terminal positions, in gum arabic (A. senegal) than in A. polyacantha.

The pattern of elimination changed in the third and fourth Smith-degradations, for which the ratio of sugars: amino acids eliminated were 12:1 and 8:1 (16:1 and 75:1 for A. polyacantha gum (91)) respectively. Considerable amounts of amino acids are therefore associated with the innermost arabinose units and those galactose residues that resisted the first three Smith degradations. Previous interpretations (19,83) of the methylation and periodate oxidation data obtained for A. senegal gum and its degradation products concluded that SDIV is a branched galactan, to which all of

the arabinose (in chains not exceeding four residues in length), rhamnose and uronic acid residues, plus much additional galactose, are attached; these interpretations, however, did not take the presence of amino acids, peptides, polypeptides or proteins into account.

Table III.19 presents the results of the amino acid analyses for hydrolysates of gum arabic and its sequential Smith degradation products. All of the minor amounts of arginine, isoleucine, lysine, methionine and tyrosine present originally were eliminated, and the amounts of alanine, aspartic acid, glutamic acid, phenylalanine and valine were greatly reduced, in the first Smith-degradation. This suggests that these amino acids were mainly associated with the periphery of the gum arabic molecule. Hydroxyproline remained the major amino acid present in all degradation products and the relative proportions of histidine, leucine, proline, serine and threonine increased during the sequence of degradations. All of the degradation products are more proteinaceous than the whole gum and have similar amino acid compositions; hydroxyproline, proline, serine, threonine and leucine together account for 82% of their amino acid content. Table III.20 shows that SDIV retains 13% of the original hydroxyproline, 29% of the original serine and threonine, 24% of the proline, 23% of the histidine and 20% of the original leucine.

This study has revealed that gum arabic (Acacia senegal) has structural features that are broadly similar, but with minor differences, to those established for Acacia

polyacantha gum (91). The ratio of amino acids to sugars in the Smith-degradation products SDII and SDIII and in the branched galactan core of the gum (SDIV) is considerably greater than in the whole gum, and these degradation products are enriched particularly in the amino acids (proline, serine, threonine) commonly encountered in sugar-amino acid linkages in glycoproteins and glycosaminoglycans. The majority of the eliminable amino acids are associated with the periodate-vulnerable sugars intermediate between the periphery of the molecule and the branched galactan core. Because much of the amino acid content is essentially internal, the amino acid content of the gum cannot readily be reduced by mild chemical treatments nor by the action of enzymes. This explains earlier failures (69,97) to effect the elimination of nitrogenous components from *Acacia senegal* and other gum exudates; only slight reductions in the nitrogen content were achieved. Gum arabic cannot be completely deproteinised without gross degradation of the gum molecules and destruction of its functionality (e.g. emulsification) and surface activity. The precise structural role of the amino acid moieties has yet to be determined.

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CHAPTER IV

AN ANALYTICAL STUDY OF *ACACIA* GUM EXUDATES

CHAPTER IV.1. SERIES PHYLLODINEAE, SUB-SERIES 6F (UNINERVES RACEMOSAE) AND 7F (PLURINERVES NERVOSAE)

IV.1(i) INTRODUCTION

The genus Acacia (Family Leguminosae, sub-family Mimosoideae) is one of the largest in the Plant Kingdom and contains over 900 species (1,2). In Australia alone, about 729 species of Acacia have been described (3), the majority of the remaining species being indigenous to Africa and America. The Acacia genus still provides many complex botanical problems of nomenclature and classification, one of which is the continuous increase in the number of Acacia species known, making previous classification outdated and incomplete, also the continuous changes and increases in the names and 'fine structure' of sub-species and varieties (4).

The most useful botanical classification is still that of Bentham (5) who divided Acacia into six Series and fifteen sub-series, which were further divided into Sections and sub-sections by Taubert (6). Although various revisions have been necessary, Bentham's main divisions or Series are still used. Bentham's divisions, based on habit, inflorescence and geographical distribution, are: Series 1, PHYLLODINEAE, subdivided into eight sub-series; Series 2, BOTRYOCEPHALAE; Series 3, PULCHELLAE; Series 4, GUMMIFERAE, with three sub-series; Series 5, VULGARES, with four sub-series; Series 6, FILICINAE.

Species from Series 1 are native to Australia, Hawaii and New Caledonia; Series 2 and 3 are native to Australia;

Series 4 and 5 are found throughout tropical and semi-tropical parts of the world; and Series 6 are native to South America. Gum exudates from Acacia species in Series 3 and 6 have not yet been studied, but comparisons of analytical and structural data for species belonging to Series 1, 4 and 5 have been made (7) and, in general, the chemical evidence substantiates Bentham's taxonomic divisions. Chemical analyses of ca. 100 different species of Series 1, 2, 4 and 5 have now been carried out (4,7), with the conclusion that each Acacia species exudes a gum that is characteristic of that species regardless of where it is grown geographically, the chemical composition and physical properties of the exudate from each Acacia species differing, often very considerably, from that of other species.

The majority of Australian species of genus Acacia belong to Series 1, Phyllodineae, which is by far the largest and most variable Series (7). Gum exudates from species in Series 1 are quite varied but, as many native Australian genera exhibit considerable taxonomic variation, thought to be due to the isolation of Australia from other land masses (8), this is perhaps, not surprising. The large number of species in the Series Phyllodineae was subdivided by Bentham into eight sub-series, namely: Alatae, Continuae, Pungentes, Calamiformes, Brunioideae, Uninerves, Plurinerves and Juliflorae.

Analytical data for about 20 Phyllodine species, mostly belonging to Bentham's sub-series 6F (Uninerves racemosae) and 7F (Plurinerves nervosae) have been noted (9). The group of 55 species of the Uninerves racemosae is considered to be

primitive, while the Plurinerves are considered to be more advanced, chemically and morphologically (11). The section Juliflorae is complex and has been treated separately (9,10). Analytical data (9) showed that the gums of sub-series 6F and 7F are even more variable in composition than was apparent previously; e.g. nitrogen (0.08-4.1%); methoxyl (0.4-2.9%); specific rotation (-54° to +90°), intrinsic viscosity (4-39 mlg^{-1}); rhamnose (trace-23%); and ratio of galactose/arabinose (2/1-25/1).

In addition to these analytical parameters the nitrogen present in Acacia gum exudates has been shown to be proteinaceous (13) and, as knowledge of this component is one of the factors essential for a more complete understanding of their properties, biosynthesis and tertiary structures (9), the amino acid composition of gum exudates must also be determined. The protein content for Acacia gum exudates ranges from ca. 0.2-45% (12) and comparisons (12,14) have shown that the amino acid compositions of gums from Acacia species within the sub-series Uninerves racemosae can vary considerably e.g. hydroxyproline content (55 residues per 1000 residues - 287 residues per 1000 residues).

This chapter presents an analytical study of gum exudates from fourteen Acacia species belonging to Uninerves racemosae, including the amino acid composition for twelve of them. The amounts of two samples, for Acacia leptopetala BRM 4094 and A.salicina were too small in size and low in protein content to allow amino acid analysis. The gums studied are from Acacia aestivalis, A. ampliceps, A. jennerae, A. leptopetala (two samples), A. ligulata, A. meisneri (two samples),

A. microbotrya (two samples), A. pruinocarpa (two samples), A. salicina and A. victoriae. Also studied is one member of Plurinerves nervosae, A. fragilis, for both the traditional analytical parameters and for its amino acid composition. In addition, the amino acid composition for the gums of a further five members of Uninerves racemosae, A. pycnantha (two samples), A. saligna (two samples) whose analytical parameters have previously been published (12) and A. victoriae BRM 4245, have been established.

One of these samples, A. pycnantha S.A., is from South Africa, the remaining samples are all from Australia, either Western Australia or Northern Territory. In the instances where two samples of an Acacia species have been studied, these samples have been collected at different locations and are being studied to see how closely they correlate. By studying such a large number of species from one sub-series, Uninerves racemosae, it is hoped to further define the analytical parameters previously reported for this group (9, 12).

IV.1(ii) ORIGIN OF GUM SAMPLES

Gum from the following species was collected in Western Australia by B.R. Maslin (voucher specimens lodged at W.A. Herbarium, Perth): Acacia aestivalis (BRM 4092), A. fragilis (BRM 3873), A. leptopetala (BRM 4094), A. leptopetala (BRM 4166), A. meisneri (BRM 4089), A. meisneri (BRM 4091), A. microbotrya (BRM 3823), A. microbotrya (BRM 3969), A. pycnantha (BRM 3982), A. saligna (BRM 3816) and A. victoriae (BRM 4245). Gum from A. ampliceps (Ref. No. T.W.97 lodged at

Herbarium, Perth) was collected by T. Willing 18 km North of Sandfine Roadhouse, Gt. Northern Highway, Western Australia in October 1983 while that of A. pruinocarpa (W.A.) was collected by M. Blackwell in Western Australia on the 1st April 1977. Gum from the following species was collected by P.K. Latz from Napperby Station, Northern Territory, Australia; A. jennerae (Latz 5937), A. ligulata (P.K. Lot 2), and A. pruinocarpa (W.A. - no voucher). The following samples were collected near Alice Springs, Northern Territory: A. salicina (N.T. 37061) and A. victoriae (N.T. 37060), while the gum for A. saligna (N.T.) was collected by J.R. Maconochie on 19 April 1978 from trees cultivated at Alice Springs. The South African sample from A. pycnantha (S.A.) was collected near Stellenbosch, Cape Province, in November 1974 and October 1975.

IV.1(iii) PREPARATION OF GUM SAMPLES FOR ANALYSIS

For the following species the gum was prepared for analysis by first having any obvious bark or dirt removed from the samples and then being ground by a mortar and pestle and passed through a stainless steel sieve (B.S. 410) with an average aperture of 150 μm: A. aestivalis, A. ampliceps, A. jennerae, A. ligulata, A. microbotrya (two samples), A. pruinocarpa (two samples), A. pycnantha (two samples), A. saligna (two samples) and A. victoriae (two samples).

The gum from the following species was first dissolved in water, dialysed against running tap water for 48 hours, and then freeze-dried: A. fragilis, A. leptopetala (two samples), A. meisneri (two samples) and A. salicina.

IV.1(iv) RESULTS AND DISCUSSION

Analytical data for the 20 samples studied are shown in Tables IV.1-8. The samples were hydrolysed with N-Sulphuric acid and the hydrolysates were examined by paper chromatography in solvent (b). The gums all contained galactose, arabinose and rhamnose, with galactose as the major component.

From the analytical data obtained in this study, the previously reported range of parameters (9,14,15,16) for species of the sub-series 6F (Uninerves racemosae) and 7F (Plurinerves nervosae) will have to be further extended. While most of the results fall within these ranges e.g. nitrogen (0.08-4.1%); specific rotation (-54° to +90°); intrinsic viscosity (4-39 gml^{-1}); uronic acid content (5-36%); rhamnose (trace -23%); and ratio of galactose/arabinose (2/1 - 25/1), the reported methoxyl range (0.4 - 2.9%) no longer applies. Both lower, 0.21% for A. victoriae N.T. 37060 and A. salicina, and higher values, 5.37% for A. pruinocarpa N.T. and 5.47% for A. pruinocarpa W.A., have been found. A. pruinocarpa, both samples, also falls ($0.32x10^5$ and $0.36x10^5$) outwith the known range of weight average molecular weight ($0.42x10^5$ - $20x10^5$). The very high methoxyl content and high acidity of the two A. pruinocarpa gums, makes them similar to gums from species in Bentham's sub-series 8 (Juliflorae), the highest known methoxyl content (5.5%) belonging to A. tumida of that sub-series (17), however, their very low molecular weight and viscosity is not typical of that group.

It has long been established (4) that Acacia gum exudates are characteristic of a particular species, but that seasonal,

TABLE IV.1: Analytical data for gum exudates from *Acacia* species, series Phyllodineae, sub-series *Uninerves racemosae*

	A. ampliceps	*A. ligulata*	*A. pruinocarpa* N.T.	*A. pruinocarpa* W.A.	*A. salicina* W.A.	*A. victoriae* N.T. 37060
Moisture, %	14.1	10.2	12.8	11.1	12.4	8.8
Ash, %[a]	6.2	2.2	6.8	6.3	4.4	6.0
Nitrogen, %[a]	0.12	0.25	0.61	0.73	0.25	0.03
Nitrogen conversion factor (NCF)	6.46	6.54	6.52	6.73	6.25	6.38
Hence protein, % (NxNCF)[a]	0.78	1.64	3.98	4.91	1.56	0.19
Methoxyl, %[b]	1.09	1.07	5.37	5.47	0.21	0.21
Specific rotation in water (degrees)[b]	+32	-0.3	-7	-8	+47	+56
Intrinsic viscosity, $ml g^{-1}$	8.8	11.9	6.4	5.5	12.2	13.8
Molecular weight ($M_w \times 10^5$)[a]	1.4	4.2	0.36	0.32	9.1	2.6
Equivalent weight[b]	819	1,116	623	797	678	680
Hence uronic anhydride, %[b,c]	21.5	15.8	28.2	22.1	26.0	25.9
Sugar composition after hydrolysis, %						
4-O-Methylglucuronic acid[d]	6.5	6.6	28.2	22.1	1.3	1.3
Glucuronic acid	15.0	9.4	-	-	24.7	24.5
Galactose	60	52	63	65	60	65
Arabinose	13	22	10	12	10	4
Rhamnose	6	10	3	1	4	5

Notes a-d as in Table III.1.

geographical, genetic and biosynthetic factors cause slight variations in the composition of the gum nodules exuded by different trees of any one species (18). In this study two different samples from each of four species, A. leptopetala, A. meisneri, A. microbotrya and A. pruinocarpa, have been studied and show very close correlation in their analytical data, slight variations in their nitrogen contents and molecular weights being the most marked.

In order to obtain additional data to test further the validity of earlier suggestions (7) that closely related species yield closely similar gum exudates, the opportunity has been taken to study gum exudates from two slightly differing specimens of A. microbotrya, and also to compare these analytically with the gums from two closely related species, viz. A. aestivalis and A. jennerae, which are considered (Maslin, B.R., private communication) to belong to a well-defined A. microbotrya group within the Uninerves racemosae (Table IV.2). Analytical data for three other A. microbotrya specimens and for A. chrysella, also a member of this group, are available (14).

The two A. microbotrya samples are very closely matched with only a slight difference in viscosity and in molecular weight to distinguish them. The A. jennerae sample is also very similar, the main difference being a slightly lower galactose and higher arabinose content. In contrast, the gum from A. aestivalis has several distinguishing features, particularly its higher methoxyl content, more negative specific rotation, lower neutralisation equivalent, higher uronic anhydride content, and slightly lower galactose content.

TABLE IV.2: Analytical data for gum exudates from the *A. microbotrya* group of *Acacias*, series Phyllodineae, sub-series *Uninerves racemosae*

	A. aestivalis	*A. jennerae*	*A. microbotrya* BRM 3823	*A. microbotrya* BRM 3969
Moisture, %	10.7	9.9	9.8	10.7
Ash, %[a]	3.1	2.6	0.6	0.9
Nitrogen, %[a]	0.20	0.11	0.09	0.06
Nitrogen conversion factor (NCF)	6.39	6.30	6.59	6.40
Hence protein, %(NxNCF)[a]	1.28	0.69	0.59	0.38
Methoxyl, %[b]	2.49	0.62	0.70	0.72
Specific rotation in water (degrees)[b]	-20	-8	+4	+4
Intrinsic viscosity, mlg^{-1}	6.5	6.5	5.4	6.7
Molecular weight ($M_w x 10^5$)[a]	0.54	0.57	0.42	0.71
Equivalent weight[b]	1,307	2,452	2,985	2,734
Hence uronic anhydride, %[bc]	13.5	7.2	5.9	6.4
Sugar composition after hydrolysis, %				
4-O-Methylglucuronic acid	13.5	3.7	4.2	4.3
Glucuronic acid	-	3.5	1.7	2.1
Galactose	66	69	74	74
Arabinose	20	22	17	17
Rhamnose	1	2	2	2

Notes a-d as in Table III.1.

Comparisons with the data for other phyllodine species (9,14,16) indicate that the gums from A. microbotrya and A. jennerae show similarities to those from A. chrysella, A. difformis and A. mabellae, with A. pycnantha not far removed. The gum from A. retinoides is also close but distinctly more acidic. The gums from A. calamifola and A. falacta have distinct similarities. All these gums have characteristically small positive or small negative rotations, moderate nitrogen and methoxyl contents, low intrinsic viscosities, uronic anhydride and rhamnose contents, and high ratios of galactose to arabinose. The high methoxyl content of A. aestivalis is comparable with values shown (9) by the gums from A. murrayana and A. georgineae which are, however, much more acidic; in addition, A. murrayana is much more proteinaceous. There are several points of similarity between the gums of A. aestivalis and A. rubida, but A. rubida is much less acidic and has a much lower methoxyl content.

Another pair of closely related species analysed in this study is A. leptopetala (two samples) and A. meisneri (two samples), both belonging to sub-series 6F (Uninerves racemosae). The gum samples were all collected by B.R. Maslin in Western Australia. These samples are very closely similar and show characteristics of low intrinsic viscosities, uronic anhydride, nitrogen and rhamnose contents, moderate molecular weight and methoxyl contents, small negative specific rotations and high galactose to arabinose ratios (Table IV.3). A. meisneri BRM 4089, shows a slightly higher nitrogen content but the only major difference seen in this group is in A. leptopetala BRM 4166, which has a much higher molecular weight,

TABLE IV.3: Analytical data for gum exudates from two closely related *Acacia* species, series Phyllodineae, sub-series *Univerves racemosae*

	A. leptopetala BRM 4094	*A. leptopetala* BRM 4166	*A. meisneri* BRM 4089	*A. meisneri* BRM 4091
Moisture, %	8.7	9.0	8.8	9.2
Ash, %[a]	1.2	1.1	1.8	1.3
Nitrogen, %[a]	0.06	0.11	0.38	0.11
Nitrogen conversion factor (NCF)	6.25	6.76	6.84	6.55
Hence protein, % (NxNCF)[a]	0.38	0.74	2.60	0.72
Methoxyl, %[b]	0.52	0.50	0.45	0.47
Specific rotation in water (degrees)[b]	-3	-3	-10	-8
Intrinsic viscosity, mlg^{-1}	5.2	5.0	6.3	5.6
Molecular weight ($M_w x 10^5$)[a]	1.3	2.9	1.7	1.1
Equivalent weight[b]	3,078	1,965	2,826	2,421
Hence uronic anhydride, %[bc]	5.7	9.0	6.2	7.3
Sugar composition after hydrolysis, %				
4-O-Methylglucuronic acid	3.1	3.0	2.7	2.8
Glucuronic acid	2.6	6.0	3.5	4.5
Galactose	75	75	75	73
Arabinose	16	14	16	17
Rhamnose	3	trace	3	2

Notes a-d as in Table III.1.

slightly higher acidity and lower rhamnose than the rest of the group.

Thus there are renewed indications that the gum exudates from trees of a particular species show small but distinct variations in their compositions and properties, in keeping with the complexity of the genus itself and the complexity of typical gum molecules. For some species, only marginal differences in external morphological characters are detectable by an experienced fieldsman; for other species the degree of differentiation is more extensive, leading to the recognition either of complexes or of distinct sub-species. The extent of the differences in gum composition shown by some sub-species has been studied (19,20).

For species recognised as being closely related in terms of their morphological characters, it is clear that some may yield closely similar gums (e.g. A. microbotrya and A. jennerae, A. meisneri and A. leptopetala) whilst the gums from other apparently closely related species (e.g. A. microbotrya and A. aestivalis) differ considerably (Tables IV.2,3). The constant reshuffling of genes and other inherited factors, particularly for Australian species, which lead to minor differences in some external characters, may, however, lead to more pronounced changes in secondary metabolic products so that some species long regarded as being closely related (e.g. A. pycnantha and A. saligna (syn. A. cyanophylla)) yield gum exudates that differ extensively in composition.

A. fragilis is the only member of the sub-series 7F (Plurinerves nervosae) whose gum exudate has been analysed in this study (Table IV.8). Many of the characteristics of this

sample, moderate intrinsic viscosity and methoxyl content, strong negative rotation, high molecular weight, uronic acid and rhamnose contents and low arabinose content make it very similar to gums studied (9) from A. implexa and A. cyclops, which are also members of sub-series 7F. However, both of these gums are much lower in nitrogen content. Only one member, A. victoriae NT 37060, of the sub-series 6F studied, has a gum exudate with as low an arabinose content as A. fragilis and one other, A. ligulata, has almost as high a rhamnose content as A. fragilis. A. fragilis also has the highest molecular weight (10×10^5) recorded in this study, although that of A. salicina (9.1×10^5) was also fairly high.

It has long been established (18) that one of the variable analytical parameters for gum exudates is their nitrogenous content; that the nitrogenous component is associated with high molecular weight fractions (21); that the nitrogenous component is almost completely associated (from nitrogen recovery data) with proteinaceous material (13) whose separation from the polysaccharide components is not readily achievable for some, but not all, Acacia gums (18,22); and that the properties of Acacia gum solutions are best understood in terms of the involvement of their nitrogenous components (23).

The amino acid compositions for the gums from seventeen Acacia species from sub-series 6F and one member of sub-series 7F, A. fragilis, are now compared (Tables IV.4-8). In addition to the differences in nitrogen content shown by these gums (0.02-0.65%N), the virtually complete recovery of the nitrogen content as amino acids after drastic hydrolysis of

TABLE IV.4: Amino acid composition of gum exudates from *Acacia* species, series Phyllodineae, sub-series *Uninerves racemosae*

	A. ampliceps	*A. ligulata*	*A. pruinocarpa* N.T.	*A. pruinocarpa* W.A.	*A. victoriae* N.T. 37060	*A. victoriae* BRM 4245
% Nitrogen	0.10	0.25	0.53	0.65	0.03	0.02
Alanine	50	69	59	67	86	79
Arginine	11	19	0	1	15	11
Aspartic acid	106	97	117	118	99	115
Cystine	2	0	3	6	0	0
Glutamic acid	46	44	54	64	45	62
Glycine	54	44	58	60	61	58
Histidine	55	37	59	35	36	46
Hydroxyproline	161	190	133	96	151	126
Isoleucine	20	20	20	21	20	27
Leucine	64	56	68	71	47	49
Lysine	31	25	38	42	34	50
Methionine	0	1	1	2	1	0
Phenylalanine	34	29	25	39	22	27
Proline	75	77	75	125	69	83
Serine	137	132	127	102	156	115
Threonine	57	57	60	51	78	68
Tyrosine	24	28	28	24	19	21
Valine	74	69	74	80	63	64

the proteinaceous matter has revealed that these nitrogen values reflect proteinaceous or polypeptide contents, respectively, ranging from ca. 0.2% to ca. 5% for the species under consideration. There are also interesting differences in their amino acid compositions which has led to the calculation of individual nitrogen conversion factors (N.C.F.) based on the number of N atoms present and the molecular weights of the individual amino acids, and their concentration in the total protein content of the gums. Where the amino acid composition of the gum is not known a standard N.C.F. of 6.25 is used. The concentrations of some amino acids remain reasonably constant (Tables IV.4-8) for all the gums studied (e.g. cystine, isoleucine, methionine, threonine, tyrosine and valine) while others differ widely, the most extreme case being hydroxyproline (55-272 residues per 1000 residues).

In Table IV.4 the amino acid composition of all the samples is very similar, the main differences being in the range of hydroxyproline and serine concentrations. The two A. pruinocarpa species contain virtually no arginine, while A. pruinocarpa W.A. also differs in having a much higher concentration of proline.

Within the A. microbotrya group (Table IV.5) there is close correspondence between A. aestivalis and the two A. microbotrya samples, with only the hydroxyproline content varying to any considerable extent, while A. jennerae is slightly different with lower aspartic acid, glutamic acid, lysine and phenylalanine contents and higher alanine and proline contents than the other gums. This is in direct contrast to the previous analytical data discussed for this

TABLE IV.5: Amino acid composition of gum exudates from the microbotrya group of Acacias, series Phyllodineae, sub-series Uninerves racemosae

	A. aestivalis	A. jennerae	A. microbotrya BRM 3823	A. microbotrya BRM 3969
% Nitrogen	0.18	0.11	0.11	0.05
Alanine	78	100	82	84
Arginine	11	22	18	17
Aspartic acid	124	90	106	128
Cystine	1	9	0	2
Glutamic acid	51	34	54	52
Glycine	74	62	60	61
Histidine	42	36	16	38
Hydroxyproline	55	135	134	99
Isoleucine	42	31	33	33
Leucine	71	58	62	62
Lysine	57	29	48	44
Methionine	6	5	0	7
Phenylalanine	42	15	27	30
Proline	75	121	84	80
Serine	93	87	112	102
Threonine	75	72	67	72
Tyrosine	24	19	27	19
Valine	78	79	71	72

group (Table IV.2) where A. jennerae corresponded more closely to the two A. microbotrya samples than A. aestivalis.

Due to a low % nitrogen content and the small amount of sample available, only one of the A. leptopetala samples was analysed for its amino acid content. This sample, A. leptopetala BRM 4166, was found to match very closely the amino acid profile of A. meisneri BRM 4089 (Table IV.6). The amino acid profile of A. meisneri BRM 4091 was also very similar but differed in having an appreciable arginine content and a much lower aspartic acid and slightly higher hydroxyproline content, than the other two samples from this group.

Of the Acacia gum species studied to date, amongst the most widely differing in chemical composition are A. pycnantha and A. saligna (syn. A. cyanophylla), both of which are members of sub-series 6F and have been classified by Bentham as being closely similar botanical species. The reason for the wide differences between A. pycnantha and A. saligna gums remains to be established and it is of interest to see if another chemotaxonomic marker, their amino acid composition, also differs. Table VI.7 shows that there are also considerable variations between the amino acid compositions of gums from A. pycnantha and A. saligna with differences in concentrations of alanine, cystine, leucine, lycine, serine and, especially, hydroxyproline being the most marked. A. saligna BRM 3816 has the largest concentration of hydroxyproline of all the gums in this study, and the lowest aspartic acid concentration. Both samples of A. saligna have higher hydroxyproline concentrations than appear to be characteristic of members of the Uninerves racemosae. As a group, A. saligna

TABLE IV.6: Amino acid composition of gum exudates from two closely related *Acacia* species, Series Phyllodineae, sub-series *Uninerves racemosae*

	A. leptopetala BRM 4166	*A. meisneri* BRM 4089	*A. meisneri* BRM 4091
% Nitrogen	0.11	0.38	0.11
Alanine	97	90	82
Arginine	0	2	19
Aspartic acid	124	121	86
Cystine	0	0	0
Glutamic acid	51	57	45
Glycine	66	67	62
Histidine	27	17	26
Hydroxyproline	149	161	192
Isoleucine	30	20	28
Leucine	62	50	56
Lysine	20	23	21
Methionine	0	0	2
Phenylalanine	20	23	20
Proline	75	88	92
Serine	127	131	119
Threonine	69	66	68
Tyrosine	18	19	25
Valine	65	65	57

TABLE IV.7: Amino acid composition of gum exudates from two *Acacia* species, series Phyllodineae, sub-series *Uninerves racemosae*

	A. pycnantha BRM 3982	*A. pycnantha* S.A.	*A. saligna* BRM 3816	*A. saligna* N.T.
% Nitrogen	0.3	0.3	0.1	0.18
Alanine	70	92	53	59
Arginine	8	3	5	5
Aspartic acid	112	137	69	106
Cystine	9	14	1	2
Glutamic acid	40	46	38	31
Glycine	57	64	54	37
Histidine	28	28	28	29
Hydroxyproline	177	126	272	242
Isoleucine	25	33	18	21
Leucine	74	94	43	42
Lysine	38	32	17	24
Methionine	1	0	1	1
Phenylalanine	13	13	12	13
Proline	58	52	64	67
Serine	123	108	151	153
Threonine	56	49	64	63
Tyrosine	35	19	35	36
Valine	79	90	71	70

TABLE IV.8: Analytical data and amino acid composition for *Acacia fragilis* BRM 3873, series Phyllodineae, sub-series *Plurinerves nervosae*

	A. fragilis		*A. fragilis*
Moisture, %	9.8	Alanine	54
Ash, %[a]	5.7	Arginine	13
Nitrogen, %[a]	0.57	Aspartic acid	92
Nitrogen conversion factor (NCF)	6.71	Cystine	0
Hence protein, %(NxNCF)[a]	3.82	Glutamic acid	44
Methoxyl, %[b]	0.73	Glycine	52
Specific rotation in water, (degrees)[b]	-26	Histidine	24
Intrinsic viscosity, mlg^{-1}	12.7	Hydroxyproline	231
Molecular weight, ($M_w x 10^5$)[a]	10.0	Isoleucine	21
Equivalent weight[b]	608	Leucine	52
Hence uronic anhydride, %[bc]	28.9	Lysine	28
		Methionine	0
Sugar composition after hydrolysis, %		Phenylalanine	15
4-O-Methylglucuronic acid[d]	4.4	Proline	93
Glucuronic acid	24.5	Serine	136
Galactose	56	Threonine	60
Arabinose	4	Tyrosine	24
Rhamnose	11	Valine	63

Notes a-d as in Table III.1.

and A. pycnantha have the lowest phenylalanine concentrations recorded in this study. Table IV.7 also shows that there is a good correspondence between the data for the gum specimen from A. saligna collected in Northern Territory and in Western Australia; the correspondence between the South African and Western Australian specimens of A. pycnantha is not so good. In view of the geographical differences involved, the adaptations in an introduced species and the very complex nature of these natural products, it is possibly surprising that the correspondence is as close as has been found.

The amino acid composition of A. fragilis, the only species from sub-series 7F to be studied, shows no marked differences from the 6F samples and, in fact, matches closely the amino acid composition of the A. saligna samples, with their higher hydroxyproline content (Tables IV.7,8).

Tables IV.4-7 show that there are variations in the amino acid compositions of the gums from Acacia species assigned to one sub-series, particularly for those amino acids well known (24) to participate frequently in sugar-amino acid linkages in glycoproteins and proteoglycans, as was recognised (13) when amino acid data for gum exudates were first published. Before these differences can be rationalised, a knowledge of the amino acid compositions for many more gums is required. The proteinaceous content of the complex gum molecules may comprise a mixture of proteins, whose relative proportions vary for different Acacia species.

CHAPTER IV.2 SERIES PHYLLODINEAE, SUB-SERIES JULIFLORAE

IV.2(i) INTRODUCTION

A general introduction to the genus Acacia is given in Chapter IV.1 of this thesis.

Botanically, the Juliflorae is considered to be one of the largest and most complex groups of Phyllodinous wattles, occurring in both tropical and more temperate regions of Australia, Malaysia and the Pacific Islands (27). Tindale (11) has stated that the Juliflorae are regarded as the most highly evolved of the Acacia species, both morphologically and chemically. The analytical data published previously (10,26) for the Juliflorae species showed that they had interesting combinations of chemical properties that are unusual in Acacia. It was also tentatively suggested, on the basis of the limited analytical data available, that the groupings within Juliflorae, suggested by Bentham, should be reconsidered.

Species within the sub-series Juliflorae continue to provide examples of even wider ranges of values for some of the various analytical parameters for Acacia gums than have been reported previously (10,26) e.g. rhamnose content from <1 to 17%, uronic acid content from 8 to 39.7%, methoxyl content from 0.23 to 5.5% and arabinose content from 3 to 67% (25). These values stimulate interest in this complex, heterogeneous, highly evolved taxon even more deeply.

Three members of the sub-series Juliflorae have been examined in this study, A. auriculiformis, A. eriopoda and A. tumida. These species have been analysed previously

(Table IV.9) for their chemical properties. A. tumida is similar to some other members of the sub-species Juliflorae, e.g. A. torulosa and A. difficilis, in having a very low rhamnose content, negative rotation and high values for nitrogen, methoxyl and uronic acid groups. The gum from A. eriopoda has several features (high nitrogen, methoxyl values; low rhamnose content) in common with the gums from A. tumida, A. torulosa and A. difficilis, but there are major differences in the respect that A. eriopoda gum has a positive rotation and an unusually high arabinose content; its very low rhamnose content and high ratio of arabinose to galactose (67/18) is typical of gums from species in the Series Gummiferae but this ratio is much higher than has been recorded so far, e.g. 59/33 for A. nubica gum (28). The gum from A. auriculiformis differs from A. tumida and A. eriopoda in having a much lower nitrogen concentration, a strongly positive rotation, a very high molecular weight and galactose/arabinose ratio. It is very similar in composition to gums from A. mangium and A. leptostachya (26), both of which are also members of the sub-series Juliflorae.

This chapter presents the amino acid composition of the gums from the three species A. auriculiformis, A. eriopoda and A. tumida. It was hoped to discover if the amino acid compositions of highly nitrogenous species differed from those with very low nitrogenous contents.

TABLE IV.9: Analytical data for gum exudates from Acacia species, series Phyllodineae, sub-series Juliflorae

	A. auriculiformis A. (26)	A. eriopoda (25)	A. tumida A. (25)	A. tumida B. (25)
Moisture, %	13.3	5.7	3.3	10.8
Ash, %[a]	4.8	1.7	2.2	3.0
Nitrogen, %[a]	1.1	6.7	6.7	7.1
Nitrogen conversion factor (NCF)	6.65	6.80	6.55	6.55
Hence protein, % (NxNCF)[a]	7.6	45.6	43.9	46.5
Methoxyl, %[b]	1.7	1.3	4.2	5.5
Specific rotation in water (degrees)[b]	+19	+5	-48	-9
Intrinsic viscosity, mlg^{-1}	22	19	11	30
Molecular weight, ($M_w x 10^5$)[a]	19	3.6	8.3	1.7
Equivalent weight[b]	590	1,180	660	480
Hence uronic anhydride, %[bc]	29.7	15	27	37
Sugar composition after hydrolysis, %				
4-O-Methylglucuronic acid	10.2	8	25	33
Glucuronic acid	19.5	7	2	4
Galactose	58	18	44	49
Arabinose	9	67	29	14
Rhamnose	3	<1	<1	<1

Notes a-d as in Table III.1.

IV.2(ii) ORIGIN OF GUM SAMPLES

Gum from A. auriculiformis was collected by J.F.U. Zieck on 23 July 1973 in the Office gardens of the Forest Products Research Centre, Papua New Guinea. Botanical voucher specimens for this tree have been kindly authenticated by Dr. M.D. Tindale as N.S.W. 107339. The sample from A. eriopoda was collected on 25 November 1984, 15 Kilometres north-east of Broome in Western Australia, by Mr. T. Willing. Gum from A. tumida was also collected by Mr. T. Willing in Western Australia on 16 September 1984.

IV.2(iii) PREPARATION OF GUM SAMPLES FOR ANALYSIS

The gum from A. auriculiformis was dissolved in distilled water (2% solution) over 2 days. The solution was filtered through Whatman No. 41 and No. 42 filter papers, dialysed against running tap water for 2 days, refiltered and freeze-dried. For A. eriopoda and A. tumida the gum samples were prepared for analysis by first having any obvious bark or dirt removed from the sample and then being ground by mortar and pestle and passed through a stainless steel sieve (BS. 410) with an average aperture of 150 µm.

IV.2(iv) RESULTS AND DISCUSSION

The amino acid composition of these three gum samples, A. auriculiformis, A. eriopoda and A. tumida, are very similar although, again, there is a wide range of hydroxyproline contents (Table IV.10). A. eriopoda has, by far, the highest hydroxyproline content (287 residues per 1000 residues) and

TABLE IV.10: Amino acid composition of gum exudates from *Acacia* species, Series Phyllodineae, sub-series *Juliflorae*

	A. auriculiformis	*A. eriopoda*	*A. tumida*
% Nitrogen	1.1	6.70	6.65
Alanine	62	43	36
Arginine	9	18	21
Aspartic acid	117	100	94
Cystine	0	0	6
Glutamic acid	47	30	48
Glycine	46	37	66
Histidine	39	23	29
Hydroxyproline	176	287	150
Isoleucine	17	32	45
Leucine	56	44	65
Lysine	30	25	43
Methionine	trace	2	3
Phenylalanine	36	20	30
Proline	66	47	60
Serine	134	115	111
Threonine	49	55	77
Tyrosine	20	24	34
Valine	96	97	79

seems to follow the trend reported previously (12) of decreased proportions of aspartic acid, glutamic acid, leucine and lysine with increased hydroxyproline content, although it does not have a particularly high serine content, which is also mentioned in this outline. A. auriculiformis has slightly higher concentrations of alanine, aspartic acid and serine than A. eriopoda or A. tumida.

Tables IV.4, 7 and 10 show that highly nitrogenous gums, e.g. A. eriopoda and A. tumida, have amino acid compositions that correspond closely with those for species having low nitrogen contents, and, moreover, with species currently assigned to different taxonomic sub-series. Thus A. tumida, 6.65%N (Table IV.10, Juliflorae) corresponds well in its amino acid composition with A. victoriae, 0.02-0.03%N (Table IV.4, Uninerves racemosae), and A. eriopoda, 5.27%N (Table IV.10, Juliflorae) corresponds well with A. saligna, 0.1-0.18%N (Table IV.7, Uninerves racemosae). Taxonomically this is an interesting, provisional, observation; morphological affinities within Uninerves racemosae have been confirmed for some species yet affinity, in at least this chemotaxonomic parameter, is also suggested between species in separate sub-series.

CHAPTER IV.3 SERIES BOTRYOCEPHALAE, GUMMIFERAE AND VULGARES

IV.3(i) INTRODUCTION

A general introduction to the genus Acacia is given in Chapter IV.1 of this thesis.

Bentham's Series 2, the Botryocephalae, is a comparatively small group (ca. 32 species). Some species are important for tannin production but not for commercial gum production because the gums are usually dark in colour, incompletely soluble in water and with an astringent taste. Nevertheless, the gum chemistry of the Botryocephalae is interesting. Of the 32 known species, analytical data for 12 have been published (29). Their analytical parameters showed a surprisingly wide range of values and it was proposed that this Series may be characterised by the existence of at least two different structural types of gum exudate (29). The differences in composition and properties of the gums, on the basis of which assignments of species were made to proposed Groups A and B, have been described (4). Species in Group A differ greatly in composition from gums of the Phyllodineae, and some resemblances to gum exudates of species in the Gummiferae are apparent; species in Group B have gum exudates that are typical of the Phyllodineae. Pedley (30) has also reported that the Botryocephalae have affinities with the Phyllodineae. Thus there are interesting possibilities of using the gum exudates as taxonomic markers.

The gum from A. deanei subsp. paucijuga whose amino acid composition is determined in this study, has analytical parameters (Table IV.11) that fall clearly within the ranges

proposed (29) for the Botryocephalae Group A. It has a low galactose/arabinose ratio and comparatively high values for all other parameters e.g. % nitrogen, intrinsic viscosity, molecular weight, and uronic acid content, with a rhamnose value of >4% and strongly negative specific rotation (20).

Bentham Series 4, Gummiferae, is a predominantly African group of Acacias, and contains 60 species (5) divided as follows: sub-series 1, Semibracteatae, 13 species; sub-series 2, Medibracteatae, 39 species; sub-series 3, Basibracteatae, 8 species. Gums of this series are characterised mainly by highly positive optical rotations and high molecular weight, with a tendency towards intermediate values of acidity and viscosity and low proportions of rhamnose; wide variations in nitrogen and methoxyl contents occur, however. A. xanthophloea, which has been studied previously (15), can be seen (Table IV.11) to follow this pattern.

Bentham Series 5, Vulgares, comprising 75 species, includes the most important source of commercial gum arabic, A. senegal, which has consequently been the subject of more chemical investigations into seasonal and geographical variation, and more structural analyses, than any other Acacia species. The main distinguishing features of gums from the Series Vulgares appear to be significant negative optical rotations, intermediate molecular weights (of the order of 0.5×10^6), and the presence of significant proportions of rhamnose.

This Chapter presents the amino acid composition of members from each of these three Series: A. deanei subsp. paucijuga, Series Botryocephalae; A. gerrardii and

A. xanthophloea, Series Gummiferae; and A. goetzei subsp. goetzei, Series Vulgares. Also determined are the analytical parameters of A. gerrardii and A. goetzei. Results for the analytical parameters of A. deanei and A. xanthophloea have been previously published (20,15).

It is hoped this data will give an indication of the range of amino acid composition to be expected across the different Series of Acacia and to ascertain whether the natural gums contain significant proportions of unusual amino acids or characteristic proportions of amino acids that might lead to the identification of particular gums.

IV.3(ii) ORIGIN OF GUM SAMPLES

Gum from A. deanei subsp. paucijuga was collected by L. Pedley at Brisbane Botanic Gardens, Queensland, in September 1968. The sample from A. gerrardii was collected in 1969 by Mr. T.H. Müller from a tree in the Botanic Garden, Salisbury, Rhodesia (now Harare, Zimbabwe). Gum from A. xanthophloea was collected in Kenya, East Africa, by Dr. J.O. Kokwaro, Botany Department, University of Nairobi, in August 1976, while that of A. goetzei subsp. goetzei was collected by Mr. Müller (details as for A. gerrardii).

IV.3(iii) PREPARATION OF GUM SAMPLES FOR ANALYSIS

Gum from A. deanei subsp. paucijuga, A. gerrardii and A. goetzei subsp. goetzei were dissolved in 1% $NaBH_4$ (31) within 24 hours. After dialysis against tap water for 48 hours and against distilled water for 24 hours, the solutions were

filtered through Whatman No.1 and No.42 papers, and freeze-dried. The A. xanthophloea sample dissolved in cold water and was first filtered, then dialysed for 48 hours against running tap water, refiltered and freeze-dried.

IV.3(iv) RESULTS AND DISCUSSION

The analytical data obtained for A. gerrardii (Table IV.11) show it to be a fairly typical member of the Series Gummiferae, with its highly positive specific rotation, intermediate values of acidity and viscosity and low proportion of rhamnose. Another characteristic of the Series Gummiferae is a high molecular weight but, unfortunately, the molecular weight of this sample could not be determined.

A. goetzei subsp. goetzei (Table IV.11) has a strongly negative specific rotation and a significant proportion of rhamnose (9%), both of which are characteristic of the Series Vulgares; unfortunately, the molecular weight could not be determined for this sample. The uronic anhydride content (23.3%) makes A. goetzei subsp. goetzei the most acidic gum found in this Series to date, the highest acidity recorded previously (32) belonged to A. mellifera subsp. detinens (21.4% uronic anhydride). The analytical parameters for the gums from these two species correlate very closely.

Thus the gum exudates analysed in this study for their amino acid composition can be taken to be fairly typical members of their Series.

The amino acid compositions of the four gums, A. deanei subsp. paucijuga, A. gerrardii, A. xanthophloea and A. goetzei subsp. goetzei, show a wide range of values although they all

TABLE IV.11: Analytical data for gum exudates from *Acacia* species, Series Botryocephalae, Gummiferae and Vulgares

	A. deanei subsp. *paucijuga* A.(20)	*A. gerrardii*	*A. xanthophloea* (15)	*A. goetzei* subsp. *goetzei*
Moisture, %	6.6	11.5	10.8	13.9
Ash, %[a]	2.8	1.8	2.7	4.7
Nitrogen, %[a]	0.76	1.86	0.10	0.89
Nitrogen conversion factor (NCF)	6.57	7.00	6.73	6.85
Hence protein, %(NxNCF)[a]	5.0	13.0	0.7	6.1
Methoxyl, %[b]	0.41	1.5	1.0	1.1
Specific rotation in water (degrees)[b]	-70	+92	+32	-38
Intrinsic viscosity, mlg^{-1}	20	9.5	11	20.9
Molecular weight, ($M_w x 10^5$)[a]	10.3	n.d.	8.6	n.d.
Equivalent weight[b]	1,175	1,065	1,212	754
Hence uronic anhydride, %[bc]	15.0	16.5	14.5	23.3
Sugar composition after hydrolysis, %				
4-O-Methylglucuronic acid	2.5	9.0	6.2	6.6
Glucuronic acid	12.5	7.5	8.3	16.7
Galactose	30	41	57	46
Arabinose	43	41	22	22
Rhamnose	12	1	6	9

Notes a-d as in Table III.1. n.d.: not determined

TABLE IV.12: Amino acid composition of gum exudates from *Acacia* species, Series Botryocephalae, Gummiferae and Vulgares

	A. deanei subsp. *paucijuga*	*A. gerrardii*	*A. xanthophloea*	*A. goetzei* subsp. *goetzei*
% Nitrogen	0.8	1.8	0.1	0.9
Alanine	39	53	50	56
Arginine	3	5	5	5
Aspartic acid	82	88	53	91
Cystine	0	0	0	0
Glutamic acid	3	52	37	56
Glycine	18	44	30	41
Histidine	70	26	39	33
Hydroxyproline	303	297	265	215
Isoleucine	16	35	22	15
Leucine	42	53	69	59
Lysine	16	26	17	31
Methionine	0	1	trace	0
Phenylalanine	10	18	23	35
Proline	68	53	82	64
Serine	161	97	172	121
Threonine	52	58	56	62
Tyrosine	32	32	9	26
Valine	85	59	70	88

have similar amounts of arginine, cystine, methionine and threonine. The correlation between the two members of the Series Gummiferae, A. gerrardii and A. xanthophloea, is not close. Although they correspond well for alanine, arginine, cystine, methionine, phenylalanine and threonine, the remaining amino acids show a fairly wide divergence. The most marked differences are for serine (97-172 residues per 1000 residues) and also for aspartic acid, hydroxyproline, proline and tyrosine. A. xanthophloea has a very low aspartic acid and tyrosine concentration and a very high serine content, these values being outwith the range previously seen for these amino acids in this Chapter. A. deanei subsp. paucijuga also has unusual features, with very low glutamic acid and glycine concentrations, and very high values for histidine and hydroxyproline; again these values are outwith the range previously seen in this Chapter for these amino acids. The hydroxyproline concentrations for A. deanei subsp. paucijuga, A. gerrardii and A. xanthophloea are all relatively high compared with previous values (Tables IV.4-8,10) with only A. saligna (both samples, Uninerves racemosae) and A. eriopoda (Juliflorae) showing comparable concentrations.

In this Chapter the amino acid profiles for 25 species of Acacia of different Series and sub-series have been established and, although there are other differences in amino acid composition, 20 of these samples have hydroxyproline and serine as their major amino acids. Therefore, although the amino acid composition could possibly be used to identify a gum exudate as Acacia, the differences may be too slight to pinpoint the actual Series of Acacia to which

the sample belonged. However, more amino acid analyses of gum exudates are needed if any impression of characteristic profiles for individual Series of Acacias is to be found and it may be that the amino acid compositions of plant gums is as important as polysaccharide parameters for chemotaxonomic purposes.

CHAPTER IV.4. DEGRADATIVE STUDIES OF HIGHLY PROTEINACEOUS *ACACIA* GUM EXUDATES

IV.4(i) INTRODUCTION

Data were presented in Chapter III.4 of this thesis for the sugar and amino acid composition of degradation products from gum arabic (*Acacia senegal*) after autohydrolysis, mild acidic hydrolysis, and ultraviolet irradiation. Further studies were undertaken to extend these results, using *Acacia* gums with different characteristics from three different Series. The gums involved in this study were from:- *Acacia eriopoda* and *A. tumida*, both members of Bentham's Series 1, Phyllodineae, sub-series *Juliflorae*; *A. gerrardii*, Bentham Series 4, Gummiferae and *A. goetzei* (Bentham Series 5, Vulgares).

A. goetzei (negative optical rotation, belonging to Series Vulgares), but not *A. gerrardii* (positive optical rotation, belonging to Gummiferae), may be sold as a highly nitrogenous type of gum arabic (33) for use as a food additive. *A. eriopoda* and *A. tumida* are very highly proteinaceous gums (25) that arise within the complex, phyllodinous sub-section *Juliflorae*. As such, they are not related to *A. senegal* and are thus not permissible as food additives. These gums offer, however, unique possibilities as model substances and as sources of higher yields of protein-rich fractions than can be expected from *A. senegal*, the much less proteinaceous source of most commercial shipments of gum arabic.

Solutions of the gums from *A. gerrardii*, *A. goetzei* and *A. tumida* (%N = 1.86, 0.89 and 7.66 respectively) were

subjected to mild acidic hydrolysis; a solution of the gum from A. eriopoda (%N = 6.70) was subjected to ultraviolet irradiation and acidic hydrolysis.

IV.4(ii) ORIGIN OF GUM SAMPLES

The origins of A. eriopoda and A. tumida are outlined in Chapter IV.2 and those of A. goetzei and A. gerrardii in Chapter IV.3 of this thesis.

IV.4(iii) PREPARATION OF GUM SAMPLES FOR ANALYSIS

Gum from A. eriopoda, A. tumida, A. goetzei and A. gerrardii dissolved in 1% $NaBH_4$ (31) within 24 hours. After dialysis, against running tap water for 48 hours and against distilled water for 24 hours, the solutions were filtered through Whatman No.1 and No.42 papers, and freeze-dried. Mild acidic hydrolysis and ultraviolet irradiation were carried out as outlined in Chapter III.4 of this thesis.

IV.4(iv) RESULTS

Table IV.13 shows the analytical data for the products obtained by mild acidic hydrolysis of the gums from A. gerrardii, A. goetzei and A. tumida: no diffusate was obtained from A. tumida gum. Table IV.14 shows the data obtained from A. eriopoda gum and its fractions resulting from (a) mild acidic hydrolysis and (b) ultraviolet degradation. Table IV.15 presents the amino acid compositions, and hence calculated nitrogen conversion factors, for the mild acidic degradation

TABLE IV.13 Analytical data for three Acacia gums and their degradation products formed after mild acidic hydrolysis

	A. gerrardii				A. goetzei				A. tumida			
	Whole gum	Degraded gum	Insoluble fraction	Diffusate	Whole gum	Degraded gum	Insoluble fraction	Diffusate	Whole gum	Degraded gum	Insoluble fraction	Diffusate
Yield, %	-	60	0.8	21	-	68	1.6	15	-	64	28	0
Loss on drying,%	11.5	9.2	n.d.	17.0	13.9	11.5	n.d.	15.3	11.0	10.0	8.2	
Ash, %[a]	1.8	1.9	n.d.	2.1	4.7	5.0	n.d.	1.1	2.6	0.4	8.1	
Nitrogen, %[a]	1.86	2.14	9.98	0.06	0.89	0.91	9.33	0.01	7.66	5.12	12.7	
.·. Nitrogen conversion factor[b]	7.00	7.26	6.58	6.81	6.85	6.77	6.74	n.d.	6.55	6.85	6.40	
Hence protein,%[a]	13	16	66	0.4	6	6	63	0.1	50	35	82	
Specific rotation, degrees[a]	+80	+78	n.d.	n.d.	-36	-31	n.d.	n.d.	-26	-8	n.d.	
Intrinsic viscosity, ml/g[a]	10	8	n.d.	n.d.	21	8	n.d.	n.d.	13	10	n.d.	
Equiv. weight[a]	1065	1010	n.d.	n.d.	755	625	n.d.	n.d.	660	630	n.d.	
Hence uronic anhydride, %[c]	16	17	n.d.	n.d.	23	28	n.d.	n.d.	27	28	n.d.	
Sugar composition (%) after hydrolysis[d]												
Glucuronic acid	16	17	n.d.	3	23	28	n.d.	3	27	28	n.d.	
Galactose	40	51	0	19	46	55	67	5	35	44	27	
Arabinose	43	31	36	76	22	12	33	65	31	27	63	
Rhamnose	1	1	64	1	9	5	0	27	3	1	10	

Footnotes: [a] Corrected for loss on drying (%); [b] From Table IV.16; [c] If all acidity arises from uronic acid groups; [d] Corrected for loss on drying (%) and protein content. n.d.=not determined.

TABLE IV.14 Analytical data for Acacia eriopoda gum and its degradation products formed after mild acidic hydrolysis and ultraviolet irradiation

	Acacia eriopoda gum	After acidic hydrolysis			After uv irradiation		
		Degraded gum	Insoluble material	Diffusate	Degraded gum	Insoluble material	Diffusate
Yield, %	-	52	6	22	64	23	1.9
Loss on drying, %	5.7	12.2	8.1	20.4	9.5	8.2	n.d.
Ash, %[a]	1.7	1.2	12.1	0.6	1.9	1.4	n.d.
Nitrogen, %[a]	6.70	7.76	11.8	2.16	6.15	10.3	3.99
∴ Nitrogen conversion factor[b]	6.80	7.08	6.68	6.90	7.02	6.50	6.70
Hence protein, %[a]	46	55	79	15	43	67	27
Specific rotation, degrees[a]	+4	-102	n.d.	n.d.	+7	n.d.	n.d.
Intrinsic viscosity, ml/g[a]	19	8	n.d.	n.d.	13	n.d.	n.d.
Equivalent weight[a]	1180	750	n.d.	1895	1040	n.d.	n.d.
Hence uronic anhydride, %[c]	15	23	n.d.	9	17	n.d.	n.d.
Sugar composition (%) after hydrolysis:-[d]							
Glucuronic acid	15	23	n.d.	9	17	n.d.	n.d.
Galactose	32	44	63	15	30	31	15
Arabinose	52	33	33	75	53	61	85
Rhamnose	1	0	4	1	0	8	0

Footnotes: [a, c, d] as in Table IV.13

[b] from Table IV.16

TABLE IV.15 Amino acid compositions for three Acacia gums and their degradation products formed after mild acid hydrolysis

	A. gerrardii				A. goetzei				A. tumida			
	whole gum	degraded gum	insoluble material	diffusate	whole gum	degraded gum	insoluble material	diffusate	whole gum	degraded gum	insoluble material	diffusate
% Nitrogen	1.86	2.14	9.98	0.06	0.89	0.91	9.33	0.01	7.66	5.12	12.75	0
Alanine	42	33	48	77	56	38	92	n.d.	38	32	47	n.d.
Arginine	12	8	18	12	5	17	7		25	19	35	
Aspartic acid	77	50	87	130	91	100	112		93	63	114	
Cystine	0	0	0	0	0	0	0		0	0	0	
Glutamic acid	49	42	77	127	56	46	99		45	26	64	
Glycine	59	33	77	131	41	34	82		53	40	81	
Histidine	25	13	32	17	33	32	29		9	9	13	
Hydroxyproline	320	457	0	0	215	270	19		184	351	18	
Isoleucine	36	23	90	64	15	22	43		45	19	70	
Leucine	52	37	110	101	59	48	102		59	33	83	
Lysine	23	19	36	36	31	23	24		43	24	55	
Methionine	3	0	0	0	0	0	0		0	0	0	
Phenylalanine	18	13	40	32	35	32	72		27	14	30	
Proline	58	53	80	142	64	63	73		69	61	76	
Serine	80	85	82	0	121	131	68		116	136	86	
Threonine	55	55	72	0	62	47	41		76	69	88	
Tyrosine	30	32	35	36	26	33	7		34	30	36	
Valine	60	47	117	95	88	65	131		85	72	97	
∴ Nitrogen Conversion Factor	7.00	7.26	6.58	6.81	6.85	6.77	6.74		6.55	6.85	6.40	

TABLE IV.16 The amino acid composition (residues per 1000 residues of the polypeptide component) of A. eriopoda gum and its degradation products from mild acidic hydrolysis and ultraviolet irradiation

	Acacia eriopoda gum	Degraded Gum		Insoluble material		Diffusate	
		acidic hydrol.	u.v. irradn.	acidic hydrol.	u.v. irradn.	acidic hydrol.	u.v. irradn.
Yield, %	-	52	64	6	23	22	1.9
% Nitrogen	6.70	7.76	6.15	11.8	10.3	2.16	3.99
Alanine	43	25	30	59	65	55	67
Arginine	18	6	12	14	26	0	18
Aspartic acid	100	58	62	103	67	155	81
Cystine	0	0	0	0	0	0	0
Glutamic acid	30	16	22	62	46	55	54
Glycine	37	22	31	71	62	61	70
Histidine	23	26	16	33	24	27	21
Hydroxyproline	287	415	376	54	157	139	157
Isoleucine	32	23	27	71	52	33	46
Leucine	44	22	33	100	71	57	70
Lysine	25	11	20	23	36	25	23
Methionine	2	0	0	0	0	0	9
Phenylalanine	20	10	17	46	34	29	30
Proline	47	44	55	57	61	61	70
Serine	115	129	115	92	104	132	99
Threonine	55	54	51	74	63	70	54
Tyrosine	24	25	23	30	28	20	32
Valine	97	113	111	111	104	81	100
∴ Nitrogen Conversion Factor	6.80	7.08	7.02	6.88	6.50	6.90	6.70

TABLE IV.17 Recoveries (%) of degradation products from mild acidic hydrolysis and ultraviolet irradiations

Recoveries	Mild acidic hydrolysis					Ultraviolet irradiation	
	Acacia gerrardii	*Acacia goetzei*	*Acacia tumida*	*Acacia eriopoda*	*Acacia senegal* (a)	*Acacia senegal* (a)	*Acacia eriopoda*
Degraded gum	60	68	64	52	47	84	64
Insoluble fraction	0.8	1.6	28	6	0.1	1.5	23
Diffusate	21	15	0	22	40	1.7	1.9
∴ Total recovery	82	84	92	80	87	87	89

Footnote: (a) Data from Chapter III.4

TABLE IV.18 Percentage of original amino acids and sugars in *Acacia* gums appearing in each fraction after degradative processes.

	Degraded gum		Insoluble fraction		Diffusate	
	Amino acids	Sugars	Amino acids	Sugars	Amino acids	Sugars
Mild acidic hydrolyses						
A. gerrardii	94%	85% Gal 50% Ara 55% Rha	5%	0% Gal <0.1% Ara 20% Rha	1%	15% Gal 50% Ara 25% Rha
A. goetzei	80%	97% Gal 44% Ara 45% Rha	19.8%	1% Gal 1% Ara 0% Rha	0.2%	2% Gal 55% Ara 55% Rha
A. tumida	49.5%	93% Gal 78% Ara 68% Rha	50.5%	7% Gal 22% Ara 32% Rha	0%	0% Gal 0% Ara 0% Rha
A. eriopoda	78%	74% Gal 35% Ara 0% Rha	13%	6% Gal 2% Ara 74% Rha	9%	20% Gal 63% Ara 26% Rha
A. senegal[(a)]	84%	62% Gal 22% Ara 1.5% Rha	3.5%	<0.1% Gal <0.1% Ara <0.1% Rha	12.5%	38% Gal 78% Ara 98% Rha
Ultraviolet irradiations						
A. senegal[(a)]	71.5%	98% Gal 94% Ara 94% Rha	15.5%	1% Gal 2% Ara 2% Rha	13%	1% Gal 4% Ara 4% Rha
A. eriopoda	63%	81% Gal 77% Ara 0% Rha	35.5%	17% Gal 18% Ara 100% Rha	1.5%	2% Gal 5% Ara 0% Rha

Footnotes: (a) Data from Chapter III.4

products from A. gerrardii, A. goetzei and A. tumida; Table IV.16 shows the corresponding data obtained for A. eriopoda gum after (a) mild acidic hydrolysis and (b) ultraviolet degradation. Table IV.17 collates the recoveries of the various degradation products. Table IV.18 shows the percentages of the sugars and the amino acids, originally present in the Acacia gums, that are located in each degradation product (as distinct from the sugar and amino acid compositions of these products). For comparative purposes, Tables IV.17 and 18 include data from the previous study of gum arabic presented in Chapter III.4 of this thesis.

IV.4(v) DISCUSSION

The results obtained from this study (Tables IV.13-18) show the differing responses of the four Acacia gums to mild acidic hydrolysis. Also A. eriopoda gum (Table IV.14) gave markedly different responses (yield, sugar composition, specific rotation, but not nitrogen content) when exposed to mild acidic and ultraviolet degradations. A. tumida gum was exceptional in giving no diffusate, but gave a significant yield (28%) of a highly proteinaceous (82%), insoluble fraction (Table IV.13), that inevitably contained all of the sugars and amino acids (Tables IV.13, 15 and 18) eliminated from the parent gum by mild acidic hydrolysis. The only other comparably high yield of an insoluble fraction was that obtained from A. eriopoda gum by ultraviolet irradiation (Tables IV.14 and 18). For all the gums studied, the insoluble fractions are highly proteinaceous (Tables IV.13 and 14).

The variability in the extent of the lability of the sugars and amino acids in the gums subjected to degradative processes is shown in Table IV.18. In A. gerrardii gum, the protein content is largely (94%) retained in the degraded gum, together with substantial proportions of the original sugars: 50% of the original arabinose is recovered in the dialysate, with 25% and 20% of the original rhamnose appearing in the diffusate and insoluble fractions respectively. The insoluble fraction contains no galactose.

In A. goetzei gum, 80% of the original protein is retained in the degraded gum, together with almost all (97%) of the galactose. The insoluble fraction contains ca. 20% of the original protein, but none of the rhamnose and only 1% of each of the original galactose and arabinose. The considerable amount (15%) of diffusate contains virtually none of the original protein or galactose, but large proportions of the original arabinose and rhamnose.

In A. tumida gum, only 49.5% of the original protein is retained in the degraded gum, but almost all (93%) of the original galactose, and substantial amounts of the original arabinose and rhamnose are retained. As there was no diffusate recovered, all of the degraded protein (50.5%) and sugars (including 32% of the original rhamnose) are located in the insoluble fraction.

In A. eriopoda gum, 78% of the original protein remains in the degraded gum with 13% and 9%, respectively, being located in the insoluble fraction and diffusate. Of the original sugars, 74% of the galactose and 35% of the arabinose remain in the degraded gum, but all of the original rhamnose

is eliminated, appearing in the insoluble fraction and in the diffusate (74% and 26%, respectively, of the original rhamnose). The complete elimination of rhamnose also occurred when A. eriopoda was degraded by ultraviolet irradiation; but by this mechanism all of the original rhamnose appears in the insoluble fraction, together with 35.5% of the original protein. These figures indicate that in A. eriopoda gum, and possibly also in A. tumida gum, some rhamnose may be closely associated with some of the amino acids.

The five Acacia gums thus studied to date, therefore, give distinct variations in the relative stabilities of their original proteinaceous and sugar components, and in the final locations of these in the insoluble fraction or in the diffusate. A. tumida gum is the most stable of all in respect to its original sugar composition, but the least stable with respect to its original protein content. The greatest protein stability is that of A. gerrardii gum. The gums from A. senegal and A. eriopoda behave comparably, with ca. 20% of the original protein and almost all of the original rhamnose being vulnerable to degradation.

With regard to the amino acid components, the general trend is for the insoluble Acacia fractions to be highly proteinaceous, and to contain substantial proportions of alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, proline, threonine and valine. The major amino acids in the diffusates are alanine, aspartic acid, glutamic acid, glycine, leucine, proline and valine. The hydroxyproline content of all diffusates and insoluble fractions arising from acidic hydrolysis is very low (except,

possibly, for A. eriopoda gum). The major effect is for the hydroxyproline and serine contents of the degraded gums to be enhanced. This substantiates similar indications, obtained previously from sequential Smith-degradations (34 and Chapter III.5 of this thesis).

Although hydroxyproline is the major amino acid in gum arabic (35) and in the majority, but not all (12), of the Acacia gums studied, the evidence from this, and from other studies (34, and Chapters III.4 and 5 of this thesis) is that it is mainly involved in the central structure of the gum molecule, and is unlikely to be functionally important in surface active phenomena such as emulsification.

These results provide some insight into some poorly understood, long-established, general industrial observations concerning commercial gum arabic, and also some structural observations concerning Acacia gums. These include (a) the remarkably fragile nature of gum arabic and Acacia solutions to heat, with resulting impairment of functionality, (b) the instability of gum arabic solutions below ca. pH 4.0 so that they are much less effective than other hydrocolloids (e.g. gums tragacanth and karaya) in the emulsification/stabilisation of acidic foods and beverages and, (c) the great decrease in molecular weight on autohydrolysis, which cannot be ascribed to the simplistic cleavage of peripheral, labile sugars in the proportions determined (21).

Now that there is evidence that gum arabic (and other Acacia exudates) contains amino acids, peptides etc, as well as sugars, that vary considerably in stability and molecular accessibility, progress may be possible towards an improved

understanding of its metabolic and immunological effects (36-38). After ingestion, gum arabic has been reported (40) to be only marginally degraded during its transit through the stomach; it is, however, very rapidly and efficiently decomposed by the natural microbiological populations in the hindgut (39,40). Nevertheless, the data obtained in this study indicates that some liberation of peripheral amino acids and sugars in gum arabic may occur at the pH of the stomach contents, within normal dietary transit times. Gum arabic, in what may be a very mildly degraded form, appears to be a highly acceptable substrate for some of the natural populations of microorganisms in the gut. Whether the known resultant re-distributions (41) in the relative numbers of the aerobic and anaerobic organisms in the caecum and colon are desirable or undesirable, and whether the low molecular weight products arising from the established microbiological degradation processes are harmful or beneficial to the host, has yet to be ascertained.

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CHAPTER V

ANALYTICAL AND DEGRADATIVE STUDIES OF *COMBRETUM* AND *PROSOPIS* GUM EXUDATES

CHAPTER V.1 THE AMINO ACID COMPOSITION OF *COMBRETUM* GUM EXUDATES

V.1(i) INTRODUCTION

The family *Combretaceae* has been divided (1) into two sub-families. One of these (*Combretoideae*) contains two tribes, of which one (*Combreteae*) contains three sub-tribes and these contain 16 genera in all. From the point of view of gum chemistry two of the three sub-tribes contain the important genera: the sub-tribe *Combretineae* (200 species) contains the genus *Combretum* and the sub-tribe *Terminaliinae* contains the genera *Anogeissus* (14 species) and *Terminalia* (150 species). The taxonomic classification of the family *Combretaceae* has been summarised (5).

The genus *Combretum* Loefl., indigenous to the tropics and sub-tropics except for Australia and the Pacific Islands, is the largest and one of the most complex in the Family *Combretaceae* (order *Myrtales*). The naming of species in *Combretum* has long been recognised (2) as a difficult problem, as illustrated by the fact that *ca*. 180 African and *ca*. 30 Asian species have been given more than 600 specific names by botanists over the years. Such is the complexity that most of the more commonly occurring species are known by several (as many as 12) synonyms, which have been listed (3).

There were several attempts at taxonomic classification in the nineteenth century. Engler and Diels (4) divided the species on a world-wide basis into 55 sections in 1899, and their classification still forms the basis for modern revisions. A revision by Exell and Stace (1) in 1966 grouped Engler and Diels' sections, and 10 sections added by other authors, into sub-genera, the major of which are sub-genus

Combretum, sub-genus Cacoucia and sub-genus Apetalanthum. In 1969, Exell and Stace (3) described 3 new sections (all African species) of the sub-genus Combretum; this brought the total number of sections in the genus to 68.

These taxonomic difficulties have arisen because the genus Combretum is a complex, heterogeneous population in which there appears to be a constant reshuffling of genes (2); a number of characters are found in nearly every combination. All that can be done is to give the "complexes" or "aggregates" of species the earliest, legitimate name available.

The Sahelian droughts in 1972-1974 led to serious shortages of gum arabic (Acacia senegal (L.) Willd.) but not of Combretum gums which became used extensively as adulterants. The resulting products were very unsatisfactory; Combretum gums have completely different characteristics and cannot be used as acceptable substitutes for gum arabic.

As a result of studies initiated at the time of the 1972-1974 Acacia shortage (5,6,7), it became established that the chemistry of the Combretum gums is also complex: their composition is much more complex than those of Acacia species and the differences that occur in composition and physico-chemical properties are also more extensive (Table V.1) than those reported for the Acacia genus (8). Combretum gums tend to be much more viscous and acidic than Acacias. Many fresh samples, and older samples that have been freshly crushed, have a marked odour of acetic acid, comparable with that of gum karaya (Sterculia species) (9). In addition to the presence of acetyl groups, Combretum gums are characterised by the presence of major amounts of galacturonic acid

and minor amounts of xylose (Table V.1), which have never been located in any of the many Acacia exudates studied (10). The presence of minor proportions of mannose in some Combretum species is also characteristic.

The gum nodules of many Combretum species are readily distinguishable from Acacia senegal nodules in external appearance. The Combretum nodules tend to be smaller and darker and the shape and surface texture of the nodules are different. These species tend to give very dark or characteristically deep red solutions. Some species, however, can give pale amber or pure white nodules but these are usually too small or too white to be typical of gum arabic. Careful visual inspection will usually reveal small inclusions of dark red colouring matter, particularly at the point where the nodule was attached to the tree. This is most frequently the case for Combretum gum from Mali, Chad and Niger; "White Mali gum" is on occasion misrepresented commercially as "gum acacia" (8). The possibility of differentiating visually between gum arabic and Combretum gum is lost when gum has been kibbled or powdered; resort to chemical analysis must then be made.

After a period (1978-1984) of adequate supplies, the disastrous droughts of 1983-1985 within the Sahelian Zone suddenly led to yet another international shortage of true gum arabic. Although Combretum gums, and gums from non-permitted genera, were again available for commercial exploitation, the regulatory position regarding food additives had become much more rigorous since 1974, when criteria of identity and purity and demands for safety evaluation of gum exudates

TABLE V.1 Analytical data for some Combretum gum exudates

	Combretum zeyheri	C. molle	C. apiculatum	C. obovatum	C. glutinosum	C. nigricans	C. erythrophyllum	C. verticillatum
Data on Crude gum:								
Loss on drying, 105°,%	12.5	11.8	12.4	12.2	13.0	10.6	12.0	15.7
Ash, 550°, %	6.2	5.0	6.3	5.3	3.5	3.0	3.6	3.5
Nitrogen, %	0.51	0.19	0.24	0.18	0.25	0.35	0.12	0.09
Acetyl groups, %	3.0	5.6	3.0	1.7	4.2	2.5	1.7	0.5
Data on purified gum:								
Recovery from crude, %	81	87	58	79	n.d.	80	78	87
Loss on drying, 105°,%	12.3	11.3	11.9	9.9	11.9	9.1	10.6	13.8
Ash, 550°, %	8.2	4.0	7.0	5.0	3.3	3.1	5.7	11.7
Nitrogen, %	0.16	0.08	0.25	0.10	0.07	0.10	0.11	0.10
Methoxyl, %	0.38	0.28	0.68	0.21	0.58	0.24	0.33	1.46
Specific rotation in water, (degrees)	+7	+2	+24	-18	-9	-43	-54	+53
Intrinsic viscosity, mlg^{-1}	163	74	188	139	75	35	110	60
Molecular weight, ($\bar{M}_w x 10^5$)	56	5.3	30	35	5.3	4.8	14	7.3
Equivalent weight	472	983	674	816	1073	1244	745	398
Hence uronic anhydride, %	37	18	26	22	16	14	24	44
Sugar composition after hydrolysis:-								
4-O-Methylglucuronic acid	2	2	4	1	3	1	2	9
Glucuronic acid	25	10	14	13	9	8	14	24
Galacturonic acid	10	6	8	8	4	5	8	11
Galactose	34	40	34	35	40	30	27	36
Arabinose	15	27	15	21	31	41	33	5
Rhamnose	14	16	17	9	13	15	16	13
Mannose	-	-	4	10	-	-	-	1
Xylose	tr	tr	4	3	tr	tr	tr	1

were introduced. The Combretum gums have never been permitted under any international regulatory system; toxicological safety evaluations have not been reported for the gum from any Combretum species. There have therefore been recent requests for the existing analytical data to be extended, because of the need to ensure that Combretum gums are not present as adulterants or contaminants in imports purporting to be gum arabic, nor in blends of hydrocolloids marketed for foodstuffs use. Although Combretum gums tend to be low in nitrogen content (6), it was felt that an analysis of their amino acid content would extend previous knowledge of the characteristics of some of the most commonly occurring Combretum gums.

The gum samples studied in this chapter were from Combretum glutinosum Perr. ex D.C. (Synonym C. leonense Engl. and Diels), C. nigricans Lepr. ex Guill. et Perr. var. elliottii (Engl. and Diels) Aubrèv., C. erythrophyllum (Burch.) Sond. (Syns. C. glomeruliflorum Sond., C. riparium Sond., C. Sonderi Gerr. ex Sond., C. ligustrifolium Engl. and Diels. ex Bak. f., C. lydenburgianum Engl. and Diels, C. salicifolium sensu Monro.) and C. verticillatum Engl. (Syns. C. collinum Fresen. spp. hypopilium (Diels) Okafor, C. hypopilinum Diels, C. kattoense Exell, and C. flaviflorum Exell).

V.1(ii) ORIGIN OF GUM SAMPLES

Gums from C. glutinosum, C. nigricans and C. verticillatum were collected in 1955 by Mr. Oseni, Western Region Department of Forestry, Ibadan, Nigeria. The gum from C. erythrophyllum

was collected in October 1970 by Mr. T. Gordon at Audley End Farm, Darwendele, nr. Salisbury, Rhodesia. Crude samples of the gums were used for analysis, after the removal of any obvious bark or dirt, and were first ground by mortar and pestle and passed through a stainless steel sieve (B.S. 410) with an average aperture of 150 μm.

V.1(iii) RESULTS AND DISCUSSION

The nitrogen contents tend to be low, but Table V.2 shows that all of the Combretum gums studied contain a wide range of amino acids, in agreement with previous data (8) (Table V.3). The amino acid compositions of Combretum gums differ greatly from those of gum arabic, which contains very high proportions of hydroxyproline (11). In contrast, Combretum gums are characterised by very low proportions or the absence of hydroxyproline, as occurs in gum karaya (12). The Combretum gums tend to contain higher proportions of alanine, aspartic acid, glutamic acid and glycine and less serine (and hydroxyproline) (Tables V.2 and 3) than gum arabic (11). Combretum verticillatum appears to be atypical (Table V.2), with much higher proportions of glutamic acid, histidine and proline and much less isoleucine, than the other Combretum gums presented here (Tables V.2 and 3). C. erythrophyllum is also unusual in having a higher serine content (Table V.2). Two other distinctive results from the previous analyses (8) are the high proline content of C. zeyheri and the high valine content of C. apiculatum.

Calculation of the factors for converting % nitrogen to % protein shows that some species e.g. C. erythrophyllum

TABLE V.2 Amino acid composition and calculated nitrogen conversion factor (NCF) of four Combretum gum exudates

	C. glutinosum	C. nigricans	C. erythrophyllum	C. verticillatum
% Nitrogen	0.04	0.11	0.08	0.01
Alanine	189	158	148	179
Arginine	18	6	7	0
Aspartic acid	115	142	126	143
Cystine	0	0	0	0
Glutamic acid	88	63	78	129
Glycine	95	94	114	129
Histidine	17	20	15	143
Hydroxyproline	0	0	16	0
Isoleucine	44	40	38	10
Leucine	67	73	52	36
Lysine	40	41	29	18
Methionine	0	0	0	0
Phenylalanine	29	27	26	12
Proline	59	70	66	95
Serine	76	71	115	40
Threonine	60	57	73	24
Tyrosine	23	43	25	0
Valine	80	96	73	42
∴ N.C.F.	6.67	6.54	6.33	5.46

TABLE V.3 Amino acid composition and calculated nitrogen conversion factor (NCF) of some Combretum gum exudates

	C. zeyheri	C. molle	C. apiculatum	C. obovatum
% Nitrogen	0.51	0.19	0.24	0.18
Alanine	92	100	99	107
Arginine	0	0	15	19
Aspartic acid	122	123	120	153
Cystine	0	0	0	6
Glutamic acid	80	106	66	71
Glycine	86	99	114	97
Histidine	75	85	40	20
Hydroxyproline	6	15	14	10
Isoleucine	41	45	45	44
Leucine	61	59	56	56
Lysine	36	34	44	48
Methionine	0	3	5	3
Phenylalanine	38	24	26	33
Proline	115	56	48	51
Serine	76	89	84	81
Threonine	76	70	70	85
Tyrosine	31	29	26	37
Valine	65	65	130	80
∴ N.C.F.	6.29	6.17	6.20	6.48

(%N x 6.33), C. zeyheri (%N x 6.29), C. molle (%N x 6.17) and C. apiculatum (%N x 6.20) have values close to the customary, nominal factor (%N x 6.25). In contrast, C. verticillatum (%N x 5.46) has an unusually low factor, a consequence of its high proportions of histidine, glycine and alanine, whereas C. glutinosum (%N x 6.67), C. nigricans (%N x 6.54) and C. obovatum (%N x 6.48) have higher factors which are comparable to those for gum arabic (old) (%N x 6.76) and gum arabic (new) (%N x 6.43) in Chapter III.2 of this thesis.

Combretum gums can therefore be differentiated from gum arabic (Acacia senegal (L.) Willd.) by means of their sugar and amino acid compositions, by the presence of galacturonic acid, xylose and acetyl groups, and by their greatly enhanced viscosity. The established foodstuffs and pharmaceutical standards of identity and purity for gum arabic are orientated towards the detection of its adulteration with sucrose, fructose, agar, starch, dextrin, gums karaya and tragacanth, and those Acacia gums (the "Wattle" group) which yield gums giving a high tannin content (8). As there is little possibility in modern practice, because of relative cost factors, of the adulteration of gum arabic with agar or with gums karaya and tragacanth, consideration should be given to a revision of the current criteria for identity and purity to ensure freedom from the presence of Combretum gums and other non-permitted exudates.

It is suggested (8), therefore, that the present criteria for the identity and purity of gum arabic (13,14) should be amended and augmented to include (a) a specified range (-27

to -32 degrees) for the specific optical rotation, to replace the current, non-specific requirement that solutions should be "slightly laevorotatory"; (b) a Kjeldahl nitrogen value (0.3-0.4%); (c) an intrinsic viscosity value (12-24 ml g^{-1}, or some corresponding Brookfield value, quoted in centipoise for a 20% solution); (d) absence of acetyl groups; (e) absence of galacturonic acid, xylose and mannose in an acidic hydrolysate (15). This increased range of tests need not be used on every occasion when gum is bought or sold, but would give desirable protection to buyers of gum in cases of dispute, to those responsible for correct labelling declarations, and to the eventual consumer. If there are to be meaningful food safety regulations, in terms of permitted lists of additives that have been evaluated toxicologically at great expense, there must be a basis for enforcement of the regulations. Permitted additives can only be regarded as safe, under specified conditions of use, when they comply with the criteria of identity and purity to which the Test Article, used in the toxicological evaluations, conformed.

CHAPTER V.2 DEGRADATIVE STUDIES OF *COMBRETUM NIGRICANS* AND *PROSOPIS ALBA* GUM EXUDATES

V.2(i) INTRODUCTION

The genus *Prosopis* Linnaeus belongs to the family Leguminosae, sub-family Mimosoideae. About forty-four species, distributed in semi-arid areas of South-west Asia, Africa and predominantly in America from Western North America to Patagonia (16) have been described. The genus includes the mesquites and screw-beans, plants which rank high among the trees and shrubs that give unique character to the desert. These species are important as range food plants having highly nutritious seeds and shoots, as bee plants and as timber, being much used for fencing, fuel and for carving (17). Some species, because of their tremendous power of invasion, have become nuisances to ranchers and are rigorously controlled.

Prosopis species exude a water-soluble gum which has been marketed in the past as "gum mesquite" and which has been used as a substitute for gum arabic, for industrial, non-food purposes, during periods of restricted trading or international market shortages (18). The *Prosopis* gums contain the same sugar residues as *Acacia* exudates (18) and are less complex than gums from other genera e.g. *Combretum*, *Terminalia*, *Anacardium* and *Grevillea* (19). This is not altogether unexpected; *Prosopis* and *Acacia* are classified botanically in the same sub-family, Mimosoideae, although in different tribes. However, as with *Combretum* exudates, "Mesquite" gum is not included in the American G.R.A.S. list nor in any other list of permitted food additives.

While the amino acid composition of Combretum gum exudates differs greatly from that of gum arabic (Chapter V.1 of this thesis), those of Prosopis exudates are remarkably similar (20). Two representative samples, Combretum nigricans and Prosopis alba, were subjected to mild acidic hydrolysis and ultraviolet irradiation and the fate of their amino acids determined in order to form a basis for comparison with those of gum arabic (Chapter III.4 of this thesis).

V.2(ii) ORIGIN OF GUM SAMPLES

The Combretum gum sample used (Combretum nigricans) has been characterised in Chapter V.1 of this thesis. Gum from Prosopis alba Grisebach (tree number 013) was sent by Dr. Peter Felker, University of California, Riverside, California on August 15th, 1978.

V.2(iii) PREPARATION OF GUM SAMPLES FOR ANALYSIS

Combretum nigricans and Prosopis alba gum samples were prepared for analysis by first having any obvious bark or dirt removed from the sample and then being ground by mortar and pestle and passed through a stainless steel sieve (B.S. 410) with an average aperture of 150 μm. A small amount of dark, insoluble matter was discarded from the solution of Combretum nigricans gum before subsequent degradation. Mild acidic hydrolysis and ultraviolet irradiation were carried out as in Chapter III.4 of this thesis.

V.2(iv) RESULTS AND DISCUSSION

Tables V.4 and 5 present the recoveries of degradation products, their amino acid compositions, and hence calculated nitrogen conversion factors, for Combretum nigricans and Prosopis alba gums respectively. On mild acidic hydrolysis, both gums behave similarly to gum arabic in forming a small amount of insoluble precipitate, which is much more highly proteinaceous than the parent gum, and a large, low molecular weight fraction containing very little protein, in addition to the residual, degraded gum. On ultraviolet irradiation, degradation is much less drastic; both gums form only small amounts of insoluble and diffusate fractions. Prosopis alba gum behaves very similarly to gum arabic in having higher nitrogen levels in these fractions, but this enrichment is not evident for Combretum nigricans gum.

Tables V.4 and 5 show, for each of the degradation processes, the proportions of the original amino acids that remain in the degraded gums, and also the proportions of the amino acids that are eliminated and hence appear either in the insoluble precipitates or in the diffusates. Table V.5 shows that Prosopis alba gum behaves very similarly to gum arabic in having alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine and valine as the major components of the insoluble fractions; aspartic acid, glutamic acid, glycine, proline, serine and valine are major components of the diffusates. The main difference evident between P. alba gum and gum arabic is in the residual gums - for P. alba gum only the hydroxyproline levels are enriched in the residual gum whereas, with gum arabic, serine and threonine levels were also higher.

TABLE V.4 Amino acid composition (residues per 1000 residues) for Combretum nigricans gum and its degradation products formed after mild acidic hydrolysis and ultraviolet irradiation

	Combretum nigricans						
	Whole gum	after acidic hydrolysis			after ultraviolet irradiation		
		degraded gum	insoluble fraction	diffusate	degraded gum	insoluble fraction	diffusate
% total yield (weight)	-	37	1.1	42	76	5	0.5
% nitrogen	0.10	0.10	2.40	0.05	0.10	0.11	0.07
Alanine	158	162	113	184	161	127	
Arginine	6	0	10	0	5	24	
Aspartic acid	142	107	116	231	157	63	
Cystine	0	0	0	0	0	0	
Glutamic acid	63	64	70	75	61	66	
Glycine	93	103	104	101	93	113	
Histidine	20	20	13	24	16	43	
Hydroxyproline	0	0	0	0	0	0	
Isoleucine	40	39	42	21	35	44	
Leucine	73	75	63	67	67	71	
Lysine	41	51	7	10	46	24	
Methionine	0	0	0	0	0	0	
Phenylalanine	27	32	35	13	28	36	
Proline	70	75	135	48	68	83	
Serine	71	69	97	90	66	100	
Threonine	57	60	83	45	53	89	
Tyrosine	43	39	54	14	39	56	
Valine	96	105	59	78	104	61	
∴ Nitrogen Conversion factor	6.54	6.51	6.76	6.56	6.53	6.14	

TABLE V.5 Amino acid composition (residues per 1000 residues) for Prosopis alba gum and its degradation products formed after mild acidic hydrolysis and ultraviolet irradiation

	Prosopis alba						
	Whole gum	after acidic hydrolysis			after ultraviolet irradiation		
		degraded gum	insoluble fraction	diffusate	degraded gum	insoluble fraction	diffusate
% total yield (weight)	-	48	1.8	32	88	0.2	2.1
% nitrogen	0.99	1.26	9.80	0.20	0.90	2.90	1.17
Alanine	47	32	99	77	36	107	74
Arginine	14	10	15	9	17	16	3
Aspartic acid	71	35	82	173	60	87	132
Cystine	3	0	0	0	0	0	0
Glutamic acid	39	30	93	66	31	76	64
Glycine	43	38	85	82	40	88	86
Histidine	34	40	30	21	47	23	24
Hydroxyproline	289	381	22	55	319	61	177
Isoleucine	30	22	57	30	27	49	37
Leucine	43	31	99	65	38	84	46
Lysine	24	24	16	15	31	36	15
Methionine	2	0	9	4	2	8	0
Phenylalanine	22	17	49	35	25	36	15
Proline	92	99	74	99	77	65	86
Serine	101	108	81	97	100	94	108
Threonine	53	50	72	59	56	64	45
Tyrosine	35	28	48	40	40	21	7
Valine	60	55	70	73	55	85	81
∴ Nitrogen Conversion factor	6.77	6.77	6.71	6.85	6.36	6.48	6.74

The fate of the amino acids present in Combretum nigricans gum does not follow the same degradative patterns as P. alba gum or gum arabic. Table V.4 shows that, apart from few exceptions, the distribution of amino acids remains fairly constant in all three fractions from both degradative processes, and is similar to that found in the whole gum. Unfortunately, due to a low nitrogen content (0.07%) and low yield (0.5%), amino acid analysis was not possible on the diffusate fraction of C. nigricans gum after ultraviolet irradiation. A slight enrichment of lysine and valine in the degraded gum was evident after both degradative procedures, and higher proportions of proline, serine, threonine and tyrosine occur in the insoluble fractions. The difference in behaviour of C. nigricans gum may be associated with the complete absence of hydroxyproline in the whole gum.

Although the Combretum genus is very complex (2) and results for one species cannot be taken as conclusive, it may be that the amino acids involved in Combretum gums are associated with the sugar residues within the overall macromolecular structure in a different manner to that in the Acacia and Prosopis gums, but the presence of amino acids in all three degradative fractions indicates that some are more labile and/or occupy more peripheral positions than others.

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THE GUM EXUDATES FROM SOME CLOSELY RELATED *ACACIA* SPECIES OF THE SUBSERIES *UNINERVES RACEMOSAE* (SECTION *PHYLLODINEAE*)*

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Key Word Index—*Acacia*; *Phyllodineae*; Leguminosae; gum exudates; chemotaxonomy; amino acids.

Abstract—Australian gum specimens from *Acacia aestivalis*, *A. chrysella*, *A. jennerae* and *A. microbotrya* (five specimens differing slightly in some morphological characters) have been studied. These species, placed within Bentham's Series 1, subseries 6F (*Uninerves racemosae*) are closely related, forming part of the recognized *A. microbotrya* group. The five specimens from *A. microbotrya* show minor variations, similar in extent to those established previously for gums from other species. The gums from *A. chrysella* and *A. jennerae* are similar to those from *A. microbotrya* in chemical composition. The gum from *A. aestivalis* differs from those from *A. microbotrya*, *A. chrysella* and *A. jennerae* in two main respects: it is more acidic and has a much higher methoxyl content. Thus significant differences in gum composition can be shown by some species that differ only slightly in morphological characters. Data for the amino acid compositions of the proteinaceous components of the gums from *A. aestivalis*, *A. jennerae* and *A. microbotrya* differ considerably from those for the gums from other species belonging to the *Uninerves racemosae*, e.g. *A. saliciformis* and *A. xanthina*, which are much more viscous and have higher proteinaceous contents containing much higher proportions of the amino acids commonly involved in linkages with sugars. Of the closely related species studied, *A. aestivalis* is closer to *A. microbotrya* than *A. jennerae* in terms of the amino acid compositions of their gums, a reversal in the relative affinities shown by their polysaccharide parameters. Thus amino acid compositions are of interest chemotaxonomically and also in terms of the tertiary structures of *Acacia* gum exudates.

INTRODUCTION

The section *Phyllodineae* is by far the largest and most variable in *Acacia* [2]. Analytical data for the gum exudates from *ca* 20 phyllodine species (excluding the *Juliflorae*) belonging mostly to Bentham's [3] subseries 6F (*Uninerves racemosae*) and 7F (*Plurinerves nervosae*) are available [4]. The data for the gums from some of the species studied recently [4] extended considerably the ranges of values established previously for the first few species of these subseries studied.

In order to obtain additional data to test further the validity of earlier suggestions [2] that closely related species yield closely similar gum exudates, the opportunity has been taken to study gum specimens from five slightly differing specimens of *A. microbotrya*, and also to compare these analytically with the gums from three closely related species, viz. *A. chrysella*, *A. jennerae* and *A. aestivalis*, which are considered (Maslin, B. R., personal communication) to belong to a well-defined *A. microbotrya* group within the *Uninerves racemosae*. Although some data have been published for *A. microbotrya* gum [5], the full range of analytical parameters was not determined.

As knowledge of the proteinaceous components of gum exudates is one of the factors essential for a more complete understanding of their properties, biosynthesis and tertiary structures [4], the amino acid compositions of the gums from *A. aestivalis*, *A. jennerae* and *A. microbotrya* have been determined.

RESULTS AND DISCUSSION

The analytical data obtained are shown in Tables 1 and 2. The samples of gum from the five slightly different specimens of *A. microbotrya* have similar analytical parameters, yet they are not identical, in support of their collector's ability to discern slight morphological differences. It has long been established [6] that *Acacia* gum exudates are characteristic of a particular species, but that seasonal, geographical, genetic and biosynthetic factors cause slight variations in the composition of the gum nodules produced by different trees of any one species [7]. For *A. microbotrya*, the differences shown by five specimens (Table 1) are similar in extent to those established for other species, e.g. *A. senegal* [8, 9], *A. dealbata* [10], *A. sieberana* [10], *A. nilotica* [11] and *A. karroo* [12]. For *A. microbotrya* gum, the nitrogen content ranges from 0.06 to 0.16%; the methoxyl content from 0.43 to 0.72%; the specific rotation from +4 to −7°; the weight-average molecular weight from 42000 to 460000; the uronic anhydride content from 6 to 8.5%; the rhamnose content from a trace to 3%; and the ratio of galactose to arabinose ranges from 74:17 to 69:21. Such variations, which exceed the limits of experimental error, are not surprising for molecular structures as large and complex as typical gum molecules. Throughout polysaccharide chemistry, one of the major advances in recent years has involved the detection of irregular structural features, e.g. the presence of 'kinking' and other random sugar residues in otherwise

*Part 70 in the series "Studies of Uronic Acid Materials". For Part 69 see ref. [1].

Table 1. Analytical data for gum polysaccharides from *Acacia* species of the section *Phyllodineae*

	A. microbotrya specimen							
	A	B	C	D	E	*A. chrysella*	*A. jennerae*	*A. aestivalis*
Loss on drying, 105°, %	9.8	10.7	(10.0)	(10.0)	(10.0)	(10.0)	9.9	10.7
Total ash, 550°, %*	0.6	0.9	1.05	1.37	1.25	0.98	2.6	3.1
Nitrogen, %*	0.09	0.06	0.16	0.16	0.14	0.11	0.11	0.20
Hence protein (N × 6.25), %*	0.55	0.35	0.98	0.98	0.84	0.69	0.69	1.26
Methoxyl, %†	0.70	0.72	0.63	0.61	0.43	0.39	0.62	2.2
Sp. rotation, $[\alpha]_D$, °†	+4	+4	−5	−3	−7	+1	−8	−18
Intrinsic viscosity, ml/g†	5.4	6.7	6.6	5.9	6.6	4.7	6.5	6.5
MW × 10^5†	0.42	0.71	1.3	1.6	4.6	1.6	0.57	0.54
Neutralization equivalent (electrodialysis)	2980	2730	2110	2190	2310	2720	2450	1310
Hence uronic anhydride, %†‡	6	6.5	8.5	8	7.5	6.5	7	13.5
Sugar composition after hydrolysis, %								
4-*O*-Methylglucuronic acid§	4	4	3.5	3.5	2.5	2.5	2.5	13
Glucuronic acid	2	2.5	5	4.5	5	4	3.5	0.5
Galactose	74	74	72	69	71	72	69	66
Arabinose	17	17	19	21	21	16	22	20
Rhamnose	2	2	tr	3	tr	5	2	1

*Corrected for moisture content.
†Corrected for moisture and protein content.
‡If all acidity arises from uronic acids.
§If all methoxyl groups are located in this acid.
tr = Trace.

Table 2. The amino acid composition (residues per 1000 residues) of the proteinaceous components of *Acacia* gums from the subsection *Uninerves Racemosae*

	A. aestivalis	*A. microbotrya*	*A. jennerae*	*A. saliciformis**	*A. xanthina**
% Nitrogen	0.20	0.06	0.11	1.2	0.67
Alanine	78	84	100	38	66
Arginine	11	17	22	3	0
Aspartic acid	124	128	90	83	96
Cystine	1	2	9	1	0
Glutamic acid	51	52	34	17	45
Glycine	74	61	62	19	50
Histidine	42	38	36	42	51
Hydroxyproline	55	99	135	287	174
Isoleucine	42	33	31	14	24
Leucine	71	62	58	41	58
Lysine	57	44	29	9	32
Methionine	6	7	5	—	—
Phenylalanine	42	30	15	11	26
Proline	75	80	121	72	79
Serine	93	102	87	171	142
Threonine	75	72	72	85	61
Tyrosine	24	19	19	30	28
Valine	78	72	79	78	70

*Data from ref. [4].

regular sequences, leading to the postulation of much clearer models and more satisfactory explanations of polymer interactions and physico-chemical behaviour in solution.

The gums from *A. chrysella* and *A. jennerae* are closely similar in composition to those from *A. microbotrya*; the minor differences are the slightly lower arabinose and slightly higher rhamnose contents of *A. chrysella* gum, and the slightly higher arabinose content of *A. jennerae* gum. In contrast, the gum from *A. aestivalis* has several distinguishing features, particularly its higher methoxyl content, more negative specific rotation, lower neutralization equivalent, higher uronic anhydride content, and slightly lower galactose content.

Comparisons with the data for other phyllodine species [4, 13] indicate that the gums from *A. microbotrya*, *A. chrysella* and *A. jennerae* show similarities to those from *A. difformis* and *A. mabellae*, with *A. pycnantha* not far removed. The gum from *A. retinodes* is also close, but distinctly more acidic. The gums from *A. calamifolia* and *A. falcata* have distinct similarities. All these gums have characteristically small positive or small negative specific rotations, moderate nitrogen and methoxyl contents, low intrinsic viscosities, uronic anhydride and rhamnose contents, and high ratios of galactose to arabinose. All the other phyllodine species studied so far show the wide variability recognized at an early stage [2] and extended even further recently [4, 14]. The high methoxyl content of *A. aestivalis* gum is comparable with the values shown [4] by the gums from *A. murrayana* and *A. georginae* which are, however, much more acidic; in addition, *A. murrayana* gum is much more proteinaceous. There are several points of similarity between the gums from *A. aestivalis* and *A. rubida*, but *A. rubida* gum is much less acidic and has a much lower methoxyl content.

Thus there are renewed indications that the gum exudates from trees of a particular species show small but distinct variations in their compositions and properties, in keeping with both the complexity of the genus itself and the complexity of typical gum molecules. For some species, only marginal differences in external morphological characters are detectable by experienced fieldsmen; for other species the degree of differentiation is more extensive, leading to the recognition either of complexes or of distinct subspecies. The extent of the differences in gum composition shown by some subspecies has been studied [15, 16].

For species recognized as being closely related in terms of their morphological characters, it is clear that some may yield closely similar gums (e.g. *A. microbotrya*, *A. chrysella*, *A. jennerae*) whilst the gums from other apparently closely related species (e.g. *A. microbotrya*, *A. aestivalis*) differ considerably (Table 1). The constant reshuffling of genes and other inherited factors, particularly for Australian species, which lead to minor differences in some external characters, may, however, lead to more pronounced changes in secondary metabolic products so that some species long regarded as being closely related [e.g. *A. pycnantha* and *A. saligna* (syn. *A. cyanophylla*)] yield gum exudates that differ extensively in composition. When surprise concerning the extent of the differences shown by these two species was first expressed [17], data were available for very few *Acacia* exudates, comparisons in terms of subdivisions of the genus had not been undertaken, and the breadth of analytical parameters now known [4, 14] to be a feature of the gums from phyllodine species had not been established. Even yet it is clearly premature to draw conclusions from the data available for gum exudates as, even in the subsections most extensively studied to date (*Uninerves racemosae* and *Plurinerves nervosae*), only *ca* 25 out of an estimated total of some 300 species have been studied. Nevertheless, the

differences shown by closely related pairs of species, e.g. *A. saligna* and *A. pycnantha*, could scarcely have failed to attract reasonable, speculative comment which has served to stimulate interest and research activity. The recent additional data have shown that *A. pycnantha* remains central within a wide range of phyllodine species and that *A. saligna* gum is no longer as unique in composition as it was when first studied. The reason for the wide differences between *A. pycnantha* and *A. saligna* gums remains to be established, but now that other closely related pairs of species have shown considerable divergence in gum compositions, the need to suggest the reallocation of *A. saligna* to a different subsection of the genus is no longer justifiable. Rather than consider the data for gum exudates in isolation, it may be prudent chemotaxonomically to consider collectively the evidence, where available, for other secondary metabolic products, e.g. for flavonoids [18], amino acids [19]. Data for the amino acid compositions of the proteinaceous components of gum exudates may prove to be useful chemotaxonomic markers.

It has long been established [7] that one of the variable analytical parameters for gum exudates is their nitrogenous content; that the nitrogenous component is associated with high molecular weight fractions [20]; that the nitrogenous component is almost completely associated (from nitrogen recovery data) with proteinaceous material [21] whose separation from the polysaccharide components is not readily achievable for some, but not all, *Acacia* gums [7, 11]; and that the properties of *Acacia* gum solutions are best understood in terms of the involvement of their nitrogenous components [22]. The importance of their proteinaceous components remains central to a more complete understanding of the properties, tertiary structure, and biosynthesis of complex gum molecules [4].

The amino acid compositions for the gums from *A. aestivalis*, *A. jennerae* and *A. microbotrya* are now compared (Table 2) with those available for other species within the *Uninerves racemosae*, i.e. *A. saliciformis* and *A. xanthina* [4]. In addition to the considerable differences in nitrogen content shown by these gums (0.06–1.2%), the virtually complete recovery of the nitrogen content as amino acids after drastic hydrolysis of the proteinaceous matter has revealed that these nitrogen values reflect protein contents, respectively, ranging from *ca* 0.4 to *ca* 8% for the species under consideration. There are also interesting differences in their amino acid compositions, with the five gums (Table 2) showing reasonably constant values for some amino acids (e.g. cystine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tyrosine and valine) but widely different proportions of others. The closest correspondences are between *A. saliciformis* and *A. xanthina* (major components: hydroxyproline and serine, both in high proportions) and between *A. aestivalis* and *A. microbotrya* (major components: aspartic acid and serine, with hydroxyproline more minor, particularly for *A. aestivalis*). The amino acid composition for *A. jennerae* appears to be intermediate between these pairs, with hydroxyproline, proline and alanine as its major components. Before these differences can be rationalized, a knowledge of the amino acid compositions for many more gums is required. The proteinaceous content of the complex gum molecules may comprise a mixture of proteins, whose relative proportions vary for different *Acacia* species.

EXPERIMENTAL

Origin of gum specimens. Gum specimens from *A. microbotrya* Benth., *A. chrysella* Maiden et Blakely, *A. jennerae* Maiden and *A. aestivalis* E. Pritzel were collected in Western Australia by Mr. B. R. Maslin under the following numbers: *A. microbotrya* sample A (BRM 3823); sample B (BRM 3969); sample C (BRM 4098); sample D (BRM 4101); sample E (BRM 4124). Of these, sample A was regarded at the time of collection as being typical *A. microbotrya*, from which sample B differed very slightly; sample C was regarded as slightly atypical; sample D was secured from a number of trees and regarded as a local variant; sample E was from a tree that yielded gum unusually copiously and regarded as a northern form of *A. microbotrya*. *A. chrysella* was sent as BRM 4153; *A. jennerae* as BRM 3961 and *A. aestivalis* as BRM 4092.

Preparation of gums for analysis. The gums (1–3 g, as available) were dissolved in distilled H_2O (100 ml), with occasional stirring, for 48 hr. The solns were filtered (muslin, then Whatman No. 1 and No. 42 papers), dialysed (for 2 days *vs.* tap water; for 1 day vs. distilled water) then recovered by freeze-drying. All the gums gave pale brown solns of very low viscosity, and are unlikely to be of any commercial interest.

Analytical methods. The standard analytical methods used for the polysaccharide components have been described [13]. The amino acid analyses were carried out with an automated analyser (Rank-Hilger Chromaspek) after hydrolysis of the gum samples in boiling 6 M HCl for 20 hr in N_2.

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THE PROTEINACEOUS COMPONENTS OF THE GUM EXUDATES FROM SOME PHYLLODINOUS *ACACIA* SPECIES*

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Key Word Index—*Acacia*; Leguminosae; Phyllodineae; gum exudates; chemotaxonomy; amino acids.

Abstract—*Acacia* gum exudates are proteinaceous polysaccharides; their protein content ranges from *ca* 0.2 to 45%. The data presented show that the amino acid compositions of the gums from 12 phyllodinous species (10 from Bentham's sub-series *Uninerves racemosae*, two from sub-series *Juliflorae*) also vary considerably, particularly in respect of their hydroxyproline content (55 residues per 1000 residues in *A. aestivalis* gum, 287 residues per 1000 in *A. saliciformis* gum). The proportions of some other amino acids, e.g. alanine, aspartic acid, proline and serine also vary considerably, but the proportions of others, e.g. cystine, methionine, histidine, threonine, tyrosine and valine, are remarkably constant. The amino acid composition of gums with a very low protein content (e.g. *A. victoriae* and *A. mycrobotrya*) is similar to that for a highly proteinaceous gum (*A. tumida*). There are, however, considerable differences between the amino acid compositions of the gums from *A. saligna* and *A. pycnantha* (South African and Western Australian specimens). This strengthens previous chemotaxonomic evidence, based on the polysaccharide parameters of their gums, that these two species are not as close taxonomically as was originally believed from morphological considerations.

INTRODUCTION

Following early observations that *Acacia seyal* gum contained a nitrogenous component [2] and that nitrogen was associated with high but not with low M_r fractions of *A. senegal* gum [3], it was reported that all plant gum exudates studied previously contained varying amounts of nitrogen ranging from traces (0.04%) to 5.6% and this nitrogenous content was shown [4] to be proteinaceous. Moreover, the amino acid compositions differed extensively [4] for different plant genera, e.g. *Acacia*, *Araucaria*, *Azadirachta* and *Lannea*. These differences have been confirmed in recent studies [5] of the gums from *Brachystegia* sp. (major components lysine and proline) and from *Julbernardia globiflora* (major components alanine and glutamic acid).

Further studies of *Acacia* gums have shown [6] that several (e.g. *A. torulosa*, *A. tumida*, *A. difficilis*, *A. eriopoda*, *A. stipuligera*) but not all (e.g. *A. dimidiata*, %N = 0.26) of the section *Juliflorae* are highly nitrogenous, corresponding to protein contents of 40–50%. This confirmed and extended previous polysaccharide evidence [7] that the *Juliflorae* comprises a number of species with widely differing chemical properties; it was suggested [7] that such species might be re-grouped more meaningfully taxonomically than they are at present. Botanically, the *Juliflorae* is the most complex group of phyllodinous *Acacias* [8].

Studies were, therefore, required to extend present knowledge [9] of the variations in amino acid composition shown by species assigned to Bentham's [10] sub-series *Uninerves racemosae* and to discover if the amino acid compositions of highly nitrogenous species differed from those with very low nitrogenous contents. In addition, studies of *A. pycnantha* gum (South African and Western Australian specimens) and of *A. saligna* gum (Northern Territory and Western Australian specimens) have been made to discover if their amino acid compositions differ significantly in view of the continued chemotaxonomic interest in these species.

RESULTS

The analytical data obtained for gum specimens from *A. pruinocarpa*, *A. pycnantha* (two specimens), *A. victoriae*, *A. tumida*, *A. ligulata*, *A. difficilis* and *A. saligna* (two specimens) are compared (Table 1) with the values reported [9] for *A. aestivalis*, *A. microbotrya*, *A. jennerae*, *A. xanthina*, and *A. saliciformis*. Of these, *A. tumida* and *A. difficilis* are placed within the *Juliflorae*, all others within Bentham's [10] sub-series *Uninerves racemosae*. To provide some basis, albeit arbitrary, for systematic comparisons, the data for these 12 species are arranged in terms of their increasing content of hydroxyproline, the amino acid showing the greatest range of values.

DISCUSSION

There are over 700 phyllodinous Australian *Acacias* (infrageneric terminology in *Acacia* is confusing and complex [Maslin, B. R., personal communication] with modern authorities (e.g. Pedley, Maslin) regarding section Phyllodineae as equivalent to Bentham's [10] series *Uninerves*); therefore, the number of species for which analytical data are available is small. Nevertheless, the

*Part 73 in the series "Studies of Uronic Acid Materials". For Part 72 see ref. [1].

Table 1. The amino acid composition (residues per 1000 residues) of the proteinaceous components of some phyllodinous *Acacia* gums

				A. pycnantha								*A. saligna*		
	A. aestivalis	*A. microbotrya*	*A. pruinocarpa*	South Africa	Western Australia	*A. jennerae*	*A. tumida*	*A. victoriae*	*A.xanthina*	*A. ligulata*	*A. difficilis*	Northern territory	Western Australia	*A. saliciformis*
% Nitrogen	0.20*	0.06*	0.65	0.25	0.28	0.11*	6.5	0.03	0.67*	0.25	7.2	0.18	0.12	1.2*
Alanine	78	84	67	88	72	100	55	81	66	69	42	61	55	38
Arginine	11	17	1	6	15	22	23	13	0	19	20	3	0	3
Aspartic acid	124	128	118	147	105	90	100	107	96	97	83	93	68	83
Cystine	1	2	6	13	9	9	5	0	0	0	5	2	2	1
Glutamic acid	51	52	64	40	43	34	50	53	45	44	43	30	41	17
Glycine	74	61	60	63	58	62	62	59	50	44	55	37	64	19
Histidine	42	38	35	27	29	36	32	41	51	37	26	28	29	42
Hydroxyproline	55	99	96	134	166	135	135	138	174	190	197	257	247	287
Isoleucine	42	33	21	31	25	31	45	23	24	20	45	21	19	14
Leucine	71	62	71	91	75	58	66	47	58	56	61	44	44	41
Lysine	57	44	42	31	38	29	46	41	32	25	36	23	17	9
Methionine	6	7	2	0	2	5	2	0	0	1	2	2	0	0
Phenylalanine	42	30	39	13	13	15	35	24	26	29	29	13	12	11
Proline	75	80	125	52	59	121	77	75	79	77	67	68	62	72
Serine	93	102	102	105	119	87	98	135	142	132	107	150	162	171
Threonine	75	72	51	50	54	72	64	72	61	57	64	62	60	85
Tyrosine	24	19	24	19	36	19	30	19	28	28	28	36	36	30
Valine	78	72	80	88	82	79	75	63	70	69	91	71	74	78

*Data from ref. [9].

species studied show a wide range of values for hydroxyproline: there are indications that the serine content tends to increase and the proportions of aspartic acid, glutamic acid, leucine and lysine to decrease as the hydroxyproline content increases. Data for nearly 200 gum specimens from a wide range of genera, acquired [Anderson, D. M. W. and McNab, C. G. A., unpublished results] during the past 3 years, are currently being evaluated prior to publication.

Although nitrogen contents of less than 0.1 % have been reported [4, 9, 11] e.g. for *A. leucoclada* (0.04 %), *A. microbotrya* (0.06 %) and *A. cyclops* (0.08 %), the nitrogen content of *A. victoriae* is even lower (0.02–0.03 %). Subsequently, it was shown that this, nevertheless, does represent a proteinaceous component with an amino acid composition similar to that of species with reasonably close affinities (*A. xanthina* and *A. ligulata*).

Table 1 also shows that highly nitrogenous [6] gums (*A. tumida* and *A. difficilis*) have amino acid compositions that correspond closely with those for species having low nitrogen contents and, moreover, with species currently assigned to different taxonomic sub-series. Thus, *A. tumida* (6.5 % N) corresponds well in its amino acid composition with *A. victoriae* (0.03 %) and *A. jennerae* (0.11 % N). Also, *A. difficilis* (7.2 % N, *Juliflorae*) corresponds well with *A. ligulata* (0.25 % N, *Uninerves racemosae*). Taxonomically this is an interesting, provisional, observation; morphological affinities within *Uninerves racemosae* have been confirmed for some species yet affinities, in at least this chemotaxonomic parameter, are also suggested between species in separate sub-series.

Table 1 shows that there is a good correspondence between the data for the gum specimens from *A. saligna* collected in Northern Territory and in Western Australia; the correspondence between the South African and Western Australian specimens of the gum from *A. pycnantha* is not so good. In view of the geographical differences involved, the adaptations in an introduced species and the very complex nature of these natural products, it is possibly surprising that the correspondence is as close as has been found. Good general agreement for many polysaccharide parameters with, however, marked geographical and seasonal variations, has been recorded for the gums from several *Acacia* sp. [12, 13]. Nevertheless, the differences in the amino acid profiles for *A. pycnantha* and *A. saligna* gums are of chemotaxonomic interest. Because the polysaccharide parameters of these two species differ so widely [4, 14], it has been suggested [14] that *A. saligna* should be re-classified within the *Juliflorae*. In terms of their amino acid compositions (Table 1) *A. pycnantha* and *A. saligna* each have closer affinities with other species (e.g. *A. saligna* with *A. saliciformis*) than they have with each other. This, therefore, confirms the previous polysaccharide evidence, summarized recently [9]. Although *A. saligna* and *A. pycnantha* have long been regarded as closely related morphologically, they do have very different numbers of flowers in each capitulum [4] and, on the basis of pollen data, are not very closely related, the pollen characters suggesting [Guinet, P., personal communication] that *A. saligna* has closer affinities with *A. dentifera*. Unfortunately, the gum from *A. dentifera* has not been available for chemical investigations. According to a leading expert in the taxonomy of Australian *Acacias*, *A. saligna* cannot be conceived as belonging to any group other than the *Uninerves racemosae* [Maslin, B. R., personal communication] although *A. saligna* and *A. pycnantha*, in the light of the chemotaxonomic evidence, are no longer regarded as being as close as was formerly believed; differences in certain minor morphological characters may have been underestimated in importance in the past [Maslin, B. R., personal communication]. It has also been concluded [9] that the chemical parameters for *A. saligna* gum can no longer be regarded as being so unusual for a member of the *Uninerves racemosae* as appeared to be the case earlier [4]. Nevertheless, *A. saligna*, on the basis of both its polysaccharide and its amino acid parameters, does have close chemical similarities with some *Juliflorae* species; equally, *A. dimidiata* [7] has chemical properties that are more typical of the *Uninerves racemosae* than of the *Juliflorae*. Such conflicts between the chemically and morphologically-based affinities may yet stimulate taxonomic re-considerations of the relative affinities of at least some species.

Table 1 shows that there are variations in the amino acid compositions of the gums from *Acacia* sp. assigned to one sub-series; the differences shown for *A. aestivalis* and *A. saliciformis* are extensive, particularly for those amino acids well-known [15] to participate frequently in sugar–amino acid linkages in glycoproteins and proteoglycans, as was recognized [4] when amino acid data for gum exudates were first published. Although the presence of proteinaceous components has continued to be reported for the gums from all the genera studied within this Chemistry Department, since that data, e.g. for *Combretum* [16], *Prosopis* [17], *Anacardium* [18], *Grevillea* [19] spp., etc., little interest in this aspect of gum chemistry has been shown by other groups of workers until recently, when a nitrogen value of 0.2 % was recorded [20] for one specimen of *A. karroo* gum, in agreement with the range of values recorded [12] for 15 different specimens of *A. karroo*, which is recognized as being highly variable. The value of 44 % arabinose reported [20] for *A. karroo* gum is not untoward for a species for which a range of 20–40 % has been recorded [12]. Even more recently there has, however, been confirmation [21] that the gum from *A. senegal* is a proteinaceous polysaccharide [3, 13]. Evidence of further strengthening of interest in protein–polysaccharide interaction arises from the recognition that arabinogalactans are often attached to greater or lesser amounts of protein in plant exudates [22], that a hemicellulosic arabinoxylan–protein complex gave a high M_r fraction having a peptide moiety rich in hydroxyproline and serine [23], and that glycogen of high M_r contains nitrogen and differs structurally from its low M_r counterpart [24]. The fact that *A. senegal* gum can be fractionated into nitrogen-enriched fractions of high M_r and nitrogen-depleted fractions of low M_r has long been recognized [3].

The data reported indicate that the protein–polysaccharide interactions and/or linkages are likely to be complex and that a better understanding is essential for more fundamental considerations of the true structure of natural gum exudates; recent re-evaluations of the complex highly branched structures proposed [25, 26] for *Acacia* gums have considered solely the polysaccharide component [27, 28]. The highly proteinaceous *Acacia* gums (Table 1) will serve as excellent model compounds, but with the knowledge that the proteinaceous components vary not only in the percentage to which they are present but also in terms of their amino acid compositions, it appears likely that the proteinaceous com-

ponent is of structural importance even in those species with very low nitrogen contents.

EXPERIMENTAL

Origin of gum specimens. The origins of the specimens from *A. aestivalis*, *A. microbotrya*, *A. jennerae*, *A. xanthina* and *A. saliciformis* have been given [9], as have those for *A. tumida* and *A. difficilis* [6]. The following specimens were kindly provided by Mr. B. R. Maslin: *A. victoriae* Benth. (BRM 4245); *A. saligna* (Labill.) H. Wendl. (BRM 3816); *A. pycnantha* Benth. (BRM 3982); *A. pruinocarpa* Tindale. The samples from *A. saligna* (Northern Territory) and *A. ligulata* Cunn. were sent by (the late) Mr. J. R. Maconochie, Alice Springs, N.T.

Analytical methods. The amino acid analyses were carried out with an automated analyser (Rank–Hilger Chromaspek) after hydrolysis of the samples in boiling 6 M HCl for 20 hr under N_2.

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The identification of *Combretum* gum exudates which are not permitted food additives†

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Gum exudates from the genus *Combretum* may be used commercially for technological applications, but are not included on any of the regulatory lists of permitted food additives. Analytical data permitting their identification are therefore required to allow food law compliance to be monitored. This paper presents data, e.g. polysaccharide and amino acid compositions, nitrogen conversion factors, and some physico-chemical parameters which characterize the gum exudates from eight *Combretum* species. Some ways in which the current specification of identity and purity for gum arabic (*Acacia senegal* (L.) Willd.) could be amended to preclude its adulteration with *Combretum* gums are suggested.

Introduction

The genus *Combretum* Loefl., cosmopolitan in the tropics and sub-tropics except for Australia and the Pacific Islands, is the largest and one of the most complex in the Family *Combretaceae* (Order *Myrtales*) as illustrated by the fact that *ca.* 180 African and *ca.* 30 Asian species have been given more than 600 specific names by botanists over the years. Examples of the extensive synonymy that can arise, and a summary of the taxonomic classification of the Family *Combretaceae*, have been given (Anderson and Bell 1977).

The Sahelian droughts in 1972–1974 led to serious shortages of gum arabic (*Acacia senegal* (L.) Willd.) but not of *Combretum* gums which became used extensively as adulterants. The resulting products were very unsatisfactory; *Combretum* gums have completely different characteristics and cannot be used as acceptable substitutes for gum arabic. The lack of analytical data for the identification of *Combretum* gums at that time led to demands, from both importers and industrial users, for studies to be undertaken. Some analytical (Anderson and Bell 1977; Anderson 1978) and structural (Anderson and Bell 1976) data were published subsequently, by which time the shortages of gum arabic had ended.

After a period (1978–1984) of adequate supplies, during which commercial initiatives were taken in attempts to boost sales and recover lost markets, the disastrous droughts of 1983–1985 within the Sahelian Zone suddenly led to yet another international shortage of true gum arabic. Although *Combretum* gums, and gums from nonpermitted genera, were again available for commercial exploitation, the regulatory position regarding food additives had become much more rigorous since 1974, when criteria of identity and purity and demands for safety evaluation of gum exudates were introduced. The *Combretum* gums have never been permitted under any international regulatory system; toxicological safety evaluations have not been reported for the gum

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from any *Combretum* species. There have therefore been recent requests for the existing analytical data to be extended, because of the need to ensure that *Combretum* gums are not present as adulterants or contaminants in imports purporting to be gum arabic, nor in blends of hydrocolloids marketed for foodstuffs use. This paper contributes some data that extend previous knowledge of the characteristics of the most commonly occurring *Combretum* gums.

Experimental

Analytical methods

The standard analytical methods for gum exudates (Anderson and Stoddart 1966; Anderson and Munro 1969) were used for the determination of moisture, ash, nitrogen, methoxyl, acetyl, equivalent weight, specific rotation, limiting viscosity number and molecular weight. The chromatographic systems and hydrolysis conditions used to determine the relative proportions of both neutral and acidic sugars have been published (Anderson and Bell 1976), as have the procedures used in amino acid analyses (Anderson *et al.* 1985; Anderson 1986a, b). Calculation of the factor for converting percentage nitrogen (Kjeldahl) to percentage protein has been described (Anderson 1986b).

Origin of gum samples

Gum from *Combretum zeyheri* Sond., (syns. *C. tinctorum* Welw. ex Laws., *C. teuszii* O. Hoffm., *C. glandulosum* F. Hoffm., *C. oblungum* F. Hoffm., *C. bragae* Engl., *C. lopolense* Engl. and Diels) was collected as small, dark nodules by Mr J. H. Dick at Isenegaza Public Lands, Tabora, Tanzania. Gum from *C. molle* R. Br. (syns. include *C. velutinum* DC., *C. guenzii* Sond., *C. holosericeum* Sond., *C. splendens* Engl., *C. elipticum* Sim, *C. pretoriense* Dummer) was collected as pale coloured, medium size nodules by Mr J. H. Dick at Tabora, Tanzania. Gum from *C. apiculatum* Sond., (Syn. *C. glutinosum* Wood non Perr. ex DC.) was obtained from Bauchi, Northern Nigeria, by Mr R. G. Kenyon, Tropical Products Institute, London. Gum from *C. obovatum* F. Hoffm. (Syn. *C. lasiocarpum* sensu Exell and Garcia) was collected as large, pale coloured nodules by Mr G. E. Rweyemamu, Regional Forest Officer, Tabora, Tanzania. Gums from *C. glutinosum* Perr. ex DC. (Syn. *C. leonense* Engl. and Diels), from *C. nigricans* Lepr. ex Guill. et Perr. var. *elliottii* (Engl. and Diels) Aubrèv., and from *C. verticillatum* Engl. (Syns *C. collinum* Fresen. spp. *hypopilinum* (Diels) Okafor, *C. hypopilinum* Diels, *C. kattoense* Exell, and *C. flaviflorum* Exell) were collected by Mr Oseni, Western Region Department of Forestry, Ibadan, Nigeria. The synonymy and collection details of the gum from *C. erythrophyllum* (Burch.) Sond. have been described (Anderson and Bell 1977).

Results

The data obtained for the general and polysaccharide-based analytical parameters for these eight *Combretum* species are presented in table 1. Data for their amino acid compositions, and nitrogen conversion factors, are given in table 2. Comparative data for gum arabic are also shown in tables 1 and 2.

Discussion

The analytical data in table 1 must be added to the data published for *C. glutinosum* (syn. *leonense*) (Anderson *et al.* 1959), *C. collinum* and *C. fragrans*

Table 1. Analytical data for the gum exudates from some *Combretum* species.

	C. zeyheri	*C. molle*	*C. apiculatum*	*C. obovatum*	*C. glutinosum*	*C. nigricans*	*C. erythrophyllum*	*C. verticillatum*	*Gum arabic*
	Data on crude gum:								
Loss on drying, 105°, %	12·5	11·8	12·4	12·2	13·0	10·6	12·0	15·7	13·6
Ash, 550°, %	6·2	5·0	6·3	5·3	3·5	3·0	3·6	3·5	4·1
Nitrogen, %	0·51	0·19	0·24	0·18	0·25	0·35	0·12	0·09	0·3
Acetyl groups, %	3·0	5·6	3·0	1·7	4·2	2·5	1·7	0·5	0
	Data on purified gum:								
Recovery from crude, %	81	87	58	79	n.d.	80	78	87	n.d.
Loss on drying, 105°, %	12·3	11·3	11·9	9·9	11·9	9·1	10·6	13·8	n.d.
Ash, 550°, %	8·2	4·0	7·0	5·0	3·3	3·1	5·7	11·7	3·0
Nitrogen, %	0·16	0·08	0·25	0·10	0·07	0·10	0·11	0·10	0·3
Methoxyl, %	0·38	0·28	0·68	0·21	0·58	0·24	0·33	1·46	0·2
Sp. Rotation, $[\alpha]_D^{25}$, degrees	+7	+2	+24	−18	−9	−43	−54	+53	−30
Intrinsic viscosity, ml/g	163	74	188	139	75	35	110	60	17
Mol. wt. ($\bar{M}_w \times 10^5$)	56	5·3	30	35	5·3	4·8	14	7·3	5·8
Equiv. wt.	472	983	674	816	1073	1244	745	398	1020
Hence uronic anyhdride, %	37	18	26	22	16	14	24	44	17
	Sugar composition after hydrolysis:								
4-*O*-Methylglucuronic acid	2	2	4	1	3	1	2	9	2
Glucuronic acid	25	10	14	13	9	8	14	24	15
Galacturonic acid	10	6	8	8	4	5	8	11	0
Galactose	34	40	34	35	40	30	27	36	45
Arabinose	15	27	15	21	31	41	33	5	24
Rhamnose	14	16	17	9	13	15	16	13	14
Mannose	—	—	4	10	—	—	—	1	0
Xylose	tr	tr	4	3	tr	tr	tr	1	0

Table 2. The amino acid composition of the proteinaceous components (residues per 1000 residues) of the gum exudates from some *Combretum* species; and calculated factors for conversion of nitrogen to protein content.

	C. zeyheri	*C. molle*	*C. apiculatum*	*C. obovatum*	*C. glutinosum*	*C. nigricans*	*C. erythrophyllum*	*C. verticillatum*	*Gum arabic*
Alanine	92	100	99	107	189	158	148	179	31
Arginine	0	0	15	19	18	6	7	0	7
Aspartic acid	122	123	120	153	115	142	126	143	60
Cystine	0	0	0	6	0	0	0	0	1
Glutamic acid	80	106	66	71	88	63	78	129	36
Glycine	86	99	114	97	95	94	114	129	49
Histidine	75	85	40	20	17	20	15	143	51
Hydroxyproline	6	15	14	10	0	0	16	0	274
Isoleucine	41	45	45	44	44	40	38	10	14
Leucine	61	59	56	56	67	73	52	36	75
Lysine	36	34	44	48	40	41	29	18	26
Methionine	0	3	5	3	0	0	0	0	0
Phenylalanine	38	24	26	33	29	27	26	12	29
Proline	115	56	48	51	59	70	66	95	77
Serine	76	89	84	81	76	71	115	40	137
Threonine	76	70	70	85	60	57	73	24	77
Tyrosine	31	29	26	37	23	43	25	0	11
Valine	65	65	130	80	80	96	73	42	45
Calculated conversion factors:									
% protein = %N ×	6·29	6·17	6·20	6·48	6·67	6·54	6·33	5·46	6·60

(Anderson and Bell 1977), *C. psidioides* (Anderson 1978), and *C. hartmannianum* (Anderson and Bell 1977). Amino acid compositions are also available for these species (Anderson *et al.* 1986).

As already established for a similarly large, genetically complex genus, e.g. *Acacia* (Anderson 1978), the *Combretum* exudates show a broad range of analytical parameters. In comparison with gum arabic (*Acacia senegal* (L.) Willd.) for which extensive data are available for samples from the major producing countries (Anderson *et al.* 1983), the *Combretum* gums tend to be much more viscous and acidic. Fresh samples, or older samples that have been freshly crushed, have a marked odour of acetic acid, comparable to that of gum karaya (*Sterculia* spp.) (Anderson *et al.* 1982).

Combretum nodules are readily distinguishable from *Acacia senegal* nodules in external appearance. *Combretum* nodules tend to be small, smooth and not fissured, hard, brittle and glass-like; they tend to give very dark or characteristically deep red solutions. Some species, however, can give pale amber or pure white nodules (too small and too white to be typical gum arabic), but careful visual inspection will usually reveal small inclusions of dark red colouring matter, particularly at the point where the nodule was attached to the tree. This is most frequently the case for *Combretum* gum from Mali, Chad and Niger; 'White Mali gum' is on occasion misrepresented commercially as 'gum acacia'. The possibility of differentiating visually between gum arabic and *Combretum* gum is lost when gum has been kibbled or powdered; resort to chemical analysis must then be made.

In addition to the presence of acetyl groups, *Combretum* gums are characterized by the presence of major amounts of galacturonic acid and minor amounts of xylose, which have never been located in any of the many *Acacia* exudates studied (Anderson 1978). The presence of minor proportions of mannose in some *Combretum* species is also characteristic.

Nitrogen contents tend to be low, but table 2 shows that all of the *Combretum* gums studied contain a wide range of amino acids, in agreement with the existing data (Anderson *et al.* 1986). The amino acid compositions of *Combretum* gums differ greatly from those of gum arabic, which contains very high proportions of hydroxyproline (Anderson *et al.* 1985b). In contrast, *Combretum* gums are characterized by very low proportions or the absence of hydroxyproline, as occurs in gum karaya (Anderson *et al.* 1985a) and in the gums from some less common genera (Anderson *et al.* 1986). The *Combretum* gums tend to contain high proportions of aspartic acid and alanine, but individual species also show high proportions of proline (*C. zeyheri*); glycine and valine (*C. apiculatum*); glycine and serine (*C. erythrophyllum*) and glutamic acid, glycine and histidine (*C. verticillatum*).

Calculation of the factors for converting percentage nitrogen to percentage protein shows that some species (e.g. *C. zeyheri*, *C. molle*, *C. apiculatum* and *C. erythrophyllum*) have values close to the customary, nominal factor ($\%N \times 6{\cdot}25$). In contrast, *C. verticillatum* has an unusually low factor, a consequence of its high proportions of histidine, glycine and alanine, whereas *C. glutinosum*, *C. nigricans* and *C. obovatum* have higher factors which are comparable to that for *A. senegal* ($6{\cdot}60 \pm 0{\cdot}14$) (Anderson *et al.* 1986).

Combretum gums can therefore be differentiated from gum arabic (*Acacia senegal* (L). Willd.) by means of their sugar and amino acid compositions, by the presence of galacturonic acid, xylose and acetyl groups, and by their greatly enhanced viscosity. The established foodstuffs and pharmaceutical standards of identity and purity for gum arabic are orientated towards the detection of its adulteration with sucrose, fructose,

agar, starch, dextrin, gums karaya and tragacanth, and those *Acacia* gums (the Wattles) which yield gums having a high tannin content. As there is little possibility in modern practice, because of relative cost factors, of the adulteration of gum arabic with agar or with gums karaya and tragacanth, consideration should be given to a revision of the current criteria for identity and purity to ensure freedom from the presence of *Combretum* gums and other non-permitted exudates.

It is suggested, therefore, that the present criteria for the identity and purity of gum arabic (FAO 1978; 1982) should be amended and augmented to include (a) a specified range (−27 to −32 degrees) for the *specific* optical rotation, to replace the current trivial, non-specific requirement that solutions should be 'slightly laevorotatory'; (b) a Kjeldahl nitrogen value (0·3–0·4%); (c) an intrinsic viscosity value (12–24 ml/g, or some corresponding Brookfield value, quoted in cp for a 20% solution); (d) absence of acetyl groups; (e) absence of galacturonic acid, xylose and mannose in an acidic hydrolysate (FAO 1983). This increased range of tests need not be used on every occasion when gum is bought or sold, but would give desirable protection to buyers of gums in cases of dispute, to those responsible for correct labelling declarations, and to the eventual consumer. If there are to be meaningful food safety regulations, in terms of permitted lists of additives that have been evaluated toxicologically at great expense, there must be a basis for enforcement of the regulations. Permitted additives can only be regarded as safe, under specified conditions of use, when they comply with the criteria of identity and purity to which the Test Article, used in the toxicological evaluations, conformed.

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The amino acid composition and quantitative sugar-amino acid relationships in sequential Smith-degradation products from gum arabic (*Acacia senegal* (L.) Willd.)

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Data are presented for the amino acid composition of gum arabic, i.e. the exudate from *Acacia senegal* (L.) Willd., and for its four sequential Smith-degradation (SD) products (0·34, 0·56, 0·87, 0·90 and 0·85% N respectively). The molar polysaccharide/protein ratio in the whole gum (31:1) decreased to 11:1 in its fourth SD product which is a proteinaceous branched galactan; proline, hydroxyproline, serine, thrèonine and leucine account for 82% of its amino acids. The first SD eliminated large amounts of sugars and small amounts of amino acids (molar ratio 40:1). The second, third and fourth degradations eliminated all of the arabinose, rhamnose and uronic acid, and 87% of the galactose originally present (molar ratios for the sugars/amino acids eliminated=42:1, 12:1 and 8:1 for SD-II, SD-III and SD-IV respectively). Overall (four SD stages), 94% of the original sugars but only 85% of the amino acids was eliminated. The proteinaceous enrichment of the galactan core largely involved serine, threonine and proline, but hydroxyproline accounts for *c*.30% of the amino acid content of the whole gum, SD-III and SD-IV, and *c*.45% of SD-I and SD-II. As deduced in a recent study of the gum from the closely related species *Acacia polyacantha*, there are indications that amino acids are associated with the sugars involved in the interior of the complex molecular gum structure.

Keywords: gum arabic, sugars, amino acids, structure, Smith-degradation

Introduction

A recent paper (Anderson 1986) summarized the reasons why a better understanding of the structure of the major commercial hydrocolloids and of the role played by their amino acid components is desirable, and presented the results of calculations based on an earlier study (Anderson and Munro 1970) of the sugar components of the gum from *Acacia polyacantha*. Although gum from that origin would be legally permissible within a consignment of gum arabic (E414), it is not available in commercial tonnages and is unlikely to be present to the extent of more than a few percent in typical gum arabic shipments, which are almost exclusively derived from the major Sahelian gum-yielding tree, *Acacia senegal* (L.) Willd. An opportunity was therefore taken to apply the method of analysis applied to *Acacia polyacantha* (Anderson 1986) to a sample of gum arabic derived from *A. senegal* and used as the Test Article in toxicological/dietary studies in rodents (Ross *et al.* 1984) and in man (Ross *et al.* 1983).

Experimental

Nitrogen determinations

Nitrogen determinations were made by the Kjeldahl method.

Amino acid analyses

Full details of the hydrolysis procedure and subsequent determination of amino acids have been given (Anderson 1986).

Origin and characterization of the gum sample

The origin of the Test Article and the data derived from complete chemical characterizations of its carbohydrate (Anderson *et al.* 1983) and amino acid components (Anderson *et al.* 1985) have been given. Comparisons of the analytical data with those for other samples of gum arabic and for botanically authenticated specimens of gum from *Acacia senegal* (L.) Willd. indicated that the Test Article can be regarded for regulatory purposes as a typical sample of commercial food-grade gum arabic.

Sequential Smith-degradations

The procedure described by Goldstein *et al.* (1965) has been summarized (Anderson 1986).

Results

The yields and analytical data obtained for the sugar compositions of *Acacia senegal* gum and its sequential Smith-degradation products SD-I-SD-IV are given in tables 1 and 2. The yields shown are corrected for the small aliquots withdrawn to monitor the progress of the degradation sequences, and for the amounts used analytically to characterize each product. When the yield of SD-I from 40 g of the Test Article was found to be lower than that (69%) reported previously (Anderson and Stoddart 1966) for a different sample of *Acacia senegal* gum (not a commercial sample) the first Smith-degradation stage was repeated a further two times on portions of the Test Article. The products' SD-I were pooled; 25% was the average recovery from the three separate preparations (25, 25 and 26% respectively) of

Table 1. Relative proportions of sugars and amino acids in *Acacia senegal* gum and its Smith-degradation products.

	Yield (%)	Hence weight (g)	Nitrogen (%) and factor[a]	Hence composition of products: Polysaccharide (g)	Polysaccharide (mM)	Protein (g)	Protein (mM)	Hence ratios:- Polysaccharide : protein (g/g)	(mM/mM)
Whole gum	—	40	0·34 (×6·76)	39·1	226[b]	0·92	7·36[c]	$\frac{39·1}{0·92} = \frac{42}{1}$	$\frac{226}{7·36} = \frac{31}{1}$
SD-I	25% of 40 g	10	0·56 (×6·79)	9·62	55	0·38	3·07	$\frac{9·62}{0·85} = \frac{25}{1}$	$\frac{55}{3·07} = \frac{18}{1}$
SD-II	51·3% of 10 g	5·13	0·87 (×6·80)	4·83	27	0·30	2·41[c]	$\frac{4·83}{0·30} = \frac{16}{1}$	$\frac{27}{2·41} = \frac{11}{1}$
SD-III	62% of 5·13 g	3·18	0·90 (×6·61)	2·99	16·6	0·19	1·57[c]	$\frac{2·99}{0·19} = \frac{16}{1}$	$\frac{16.6}{1·57} = \frac{11}{1}$
SD-IV	79% of 3·18 g	2·51	0·85 (×6·52)	2·37	13·2	0·14	1·15[c]	$\frac{2·37}{0·14} = \frac{17}{1}$	$\frac{13·2}{1·15} = \frac{11}{1}$

[a] Factors for converting %N to % protein calculated from data in table 3.
[b] See table 2.
[c] See tables 3 and 4.

Table 2. Elimination of sugars and amino acids from *Acacia senegal* gum in sequential Smith-degradations.

	Molar ratios of sugars		Hence g per 100 mol	Hence sugars in product (g)	(mM)	Hence loss of sugars (g)	(mM)	Amino acids in product (g)[a]	(mM)	Hence ratios for Sugars amino acids (g/g)	Eliminated (mM/mM)
Whole gum	Gal	45	8100	18·32	102			0·92	7·36		
(40 g) 97·7%	Ara	24	3600	8·14	54						
sugars 2·3%	Rha	14	2296	5·19	32						
protein	UA[c]	17	2298	7·46	38						
Total		100	17284	39·11	226						
SD-I (10 g)	Gal	73	13140	7·27	40	11·05	62	0·38	3·07	$\frac{(39·1-9·6)}{(0·92-0·38)} = \frac{55}{1}$	$\frac{(226-55)}{7·36-3·07} = \frac{40}{1}$
(9·6 g sugars)	Ara	22	3300	1·75	12	6·39	42				
	Rha	1	164	0·09	1	5·10	31				
	UA[c]	4	776	0·41	2	7·05	36				
Total		100	17380	9·62	55	29·49	171				
SD-II	Gal	97	17460	4·71	26	2·25	13	0·30	2·41	$\frac{(9·6-4·83)}{(0·38-0·30)} = \frac{60}{1}$	$\frac{(55-27)}{(3·07-2·41)} = \frac{42}{1}$
(5·13 g)	Ara	3	450	0·12	1	1·63	11				
(4·83 g	Rha	0	0	0	0	0·09	1				
sugars)	UA	0	0	0	0	0·41	2				
Total		100	17910	4·83	27	4·38	27				
SD-III											
(3·18 g)											
(2·99 g	Gal	97	17460	2·90	16	1·81	10	0·19	1·57	$\frac{(4·83-2·99)}{(0·30-0·19)} = \frac{16}{1}$	$\frac{(27-16·6)}{(2·41-1·57)} = \frac{12}{1}$
sugars)	Ara	3	450	0·09	0·6	0·03	0·4				
Total		100	17910	2·99	16·6	1·84	10·4				
SD-IV											
(2·51 g)											
(2·37 g	Gal	100	18000	2·37	13·2	0·53	3	0·14	1·15	$\frac{(2·99-2·37)}{(0·19-0·14)} = \frac{12}{1}$	$\frac{(16·6-13·2)}{(1·57-1·15)} = \frac{8}{1}$
sugars)	Ara	0	0	0	0	0·09	0·6				
Total						0·62	3·6				

[a] See table 1

[b] See tables 3 and 4

[c] 15·5% glucuronic acid+1·5% 4-0-methylglucuronic acid in whole gum; 4% glucuronic acid in SD-I [Anderson *et al.* 1983].

SD-I. Table 3 shows the results (mol/1000 mol of the proteinaceous component) obtained from amino acid analyses of the Test Article and its Smith-degradation products SD-I–SD-IV, together with the method of calculation used to derive quantitative values for the amino acids present. Table 4 presents the values (derived as in table 3) for the amino acids in SD-I–SD-IV; the amounts (mmol) of each amino acid lost, in the four degradation stages overall, are also shown. Table 1 includes values calculated for the molar and weight/weight ratios of the polysaccharide/protein present in *Acacia senegal* gum and its degraded products. Table 2 includes the values calculated for the ratios of the sugars/amino acids eliminated at each stage of the degradation sequence. The data in table 3 permitted

Table 3. The amino acid composition of *Acacia senegal* gum and its sequential Smith-degradation products.

Amino acid	Mol. wt	Mol./ 1000 mol.[a]	∴ Relative wts present (g)	Hence wt per 0·92 g (g)[b]	Hence mmol/ 0·92 g (mM)	Mol. per 1000 mol in Smith degradation products			
						SD-I	SD-II	SD-III	SD-IV
Column	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
Ala	89	31	2·76	0·020	0·22	8	8	14	15
Arg	174	7	1·22	0·009	0·05	0	0	0	0
Asp	133	63	8·38	0·061	0·46	11	17	19	25
Cys	121	0	0	0	0	0	0	0	0
Glu	147	35	5·15	0·037	0·25	6	6	8	12
Gly	61	48	2·93	0·021	0·34	31	29	34	42
His	155	39	6·05	0·044	0·28	50	50	58	57
Hyp	131	313	41·00	0·297	2·27	454	465	335	258
Ile	131	13	4·06	0·029	0·22	0	0	0	0
Leu	131	73	9·56	0·069	0·53	65	65	82	93
Lys	146	25	3·65	0·026	0·18	0	0	0	0
Met	149	1	0·15	0·001	0·01	0	0	0	0
Phe	165	27	4·45	0·032	0·19	5	5	9	9
Pro	115	81	9·32	0·068	0·59	71	83	112	124
Ser	105	111	11·65	0·085	0·81	189	166	189	205
Thr	119	79	9·40	0·068	0·57	104	95	125	142
Tyr	181	10	1·81	0·013	0·07	0	0	0	0
Val	117	45	5·26	0·038	0·32	7	11	15	18
Totals		1001	126·80	0·918	7·36	1001	999	1000	1000

[a] From Anderson *et al.* 1985.
[b] See table 1

calculation of the nitrogen conversion factors (table 1) and hence the amounts of proteinaceous and polysaccharide components in each product.

Discussion

Table 1 shows that the molar ratio of sugars/amino acids in *Acacia senegal* gum is 31:1 and that this ratio decreased to 18:1 (SD-I) and to 11:1 (SD-II–SD-IV). Of the original polysaccharide (39·1 g) and protein (0·92 g) in 40 g of whole gum, only 2·37 g of degraded polysaccharide (a galactan) and 0·14 g of proteinaceous or peptide material remain in SD-IV. Thus 94% of the original polysaccharide but only 85% of the protein is eliminated; the degradation products become progressively more proteinaceous than the original gum. For *Acacia polyacantha*, the molar ratio of sugars/amino acids decreased from 28:1 (whole gum) to 14:1, 14:1, 12:1 and 9:1 (products SD-I–SD-IV; 96·5% of the original polysaccharide but only 89% of the protein were eliminated. Thus there is a distinct similarity in the sequence of degradative changes for both gums, the major difference being the smaller ratio for sugars/amino acids eliminated (40:1) for *A. senegal* gum than for *A. polyacantha* gum (112:1) in the first Smith degradation.

Table 2 shows the number of millimoles of sugars eliminated at each stage of the degradation sequence. The first stage eliminated 61% (6200÷102) of the galactose, 78% of the arabinose, 97% of the rhamnose and 95% of the uronic acids

Table 4. Quantitative relationships for the amino acids in *Acacia senegal* gum and its Smith-degradation products.

Amino acid	mmol in whole gum[a]	Relative proportions[b] of amino acids in SD-I (g in 0·38 g)	SD-I (mM)	SD-II (g in 0·30 g)	SD-II (mM)	SD-III (g in 0·19)	SD-III (mM)	SD-IV (g in 0·14 g)	SD-IV (mM)	Amino acids eliminated[c] (mM)	Amino acids eliminated[c] (%)
Column	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
Ala	0·22	0·002	0·02	0·002	0·02	0·003	0·02	0·002	0·017	0·203	92
Arg	0·05	0	0	0	0	0	0	0	0	0·05	100
Asp	0·46	0·005	0·04	0·005	0·03	0·004	0·03	0·004	0·029	0·431	94
Cys	0	0	0	0	0	0	0	0	0	—	—
Glu	0·25	0·003	0·02	0·002	0·01	0·002	0·01	0·002	0·014	0·236	95
Gly	0·34	0·006	0·10	0·004	0·07	0·004	0·07	0·003	0·047	0·293	86
His	0·28	0·024	0·15	0·019	0·12	0·014	0·09	0·010	0·065	0·215	77
Hyp	2·27	0·185	1·41	0·147	1·12	0·068	0·52	0·039	0·296	1·974	87
He	0·22	0	0	0	0	0	0	0	0	0·22	100
Leu	0·53	0·026	0·20	0·021	0·16	0·016	0·13	0·014	0·107	0·423	80
Lys	0·18	0	0	0	0	0	0	0	0	0·18	100
Met	0·01	0	0	0	0	0	0	0	0	0·01	100
Phe	0·19	0·003	0·02	0·002	0·01	0·002	0·01	0·002	0·010	0·18	95
Pro	0·59	0·025	0·22	0·023	0·20	0·020	0·18	0·016	0·143	0·447	76
Ser	0·81	0·062	0·56	0·042	0·40	0·030	0·29	0·025	0·235	0·575	71
Thr	0·57	0·038	0·32	0·027	0·23	0·023	0·19	0·019	0·163	0·407	71
Tyr	0·07	0	0	0	0	0	0	0	0	0·07	100
Val	0·32	0·002	0·01	0·003	0·003	0·003	0·03	0·002	0·020	0·30	94
Totals	7·36	0·381	3·07	0·297	2·40	0·189	1·57	0·138	1·146	6·214	

[a] From table 3, column (e)

[b] Calculated from table 3, columns (f-i) respectively

[c] Column j=columns (a–i); column k=columns $j/a \times \frac{100}{1}$

present originally in the gum. Thus 76% (17100÷226) i.e. 171 mmol of the original sugars but only 4·29 mmol of the original amino acids (table 2) were eliminated, a molar ratio of 40:1 (112:1 for *A. polyacantha* gum). The peripheral, periodate-vulnerable, sugars are therefore not associated with extensive amounts of amino acids.

Table 2 shows that the situation differs greatly in the subsequent degradations. Degradation of SD-I to give SD-II eliminated 35%, 92%, 100% and 100% of its galactose, arabinose, rhamnose and uronic acids respectively, corresponding to the loss of 28 mmol of sugars. The loss of amino acids was 0·66 mmol; thus 42 mmol sugars were lost per mmol amino acids. As for *A. polyacantha* gum (this ratio=14:1) there are therefore considerable amounts of amino acids associated with the sugars eliminated during the second stage of periodate oxidation.

This pattern of elimination continued in the third and fourth Smith-degradation products for which the ratios of sugars:amino acids were 11:1 and 8:1 (12:1 and 9:1 for *A. polyacantha* gum) respectively. Considerable amounts of amino acids are therefore associated with the innermost arabinose units and those galactose residues that resisted the first three Smith degradations. Previous interpretations (Anderson and Stoddart 1966; Anderson and Street 1983) of the methylation and periodate oxidation data obtained for *A. senegal* gum and its degradation products concluded that SD-IV is a branched galactan, to which all of the arabinose (in chains not exceeding four residues in length), rhamnose and uronic acid residues, plus much additional galactose, are attached; these interpretations, however, did not take the presence of amino acids, peptides, polypeptides or proteins into account.

Table 3 presents the results of the amino acid analyses for hydrolysates of gum arabic and its sequential Smith degradations, and calculations whereby the nitrogen conversion factor (N×6·76) shown in Table 1 can be derived. Similar calculations led to the corresponding nitrogen conversion factors and quantitative data quoted for SD-I–SD-IV in tables 1 and 4. The total amounts of amino acids eliminated at each stage have been utilized in tables 1 and 2. The nitrogen conversion factors for *Acacia senegal* and its four degradation products (6·76, 6·79, 6·80, 6·61 and 6·52 respectively) are slightly lower than the corresponding values for *Acacia polyacantha* gum (6·87, 6·79, 6·82, 6·99 and 7·04 respectively) (Anderson 1986).

All of the minor amounts of methionine, arginine, isoleucine, lysine, and tyrosine present originally were eliminated, and the amounts of alanine, aspartic acid, glutamic acid, phenylalanine and valine were greatly reduced, in the first Smith degradation. Hydroxyproline remained the major amino acid present in all the degradation products. The relative proportions of histidine, leucine, proline, serine and threonine increased during the sequence of degradations. All of the degradation products are more proteinaceous than the whole gum and have similar amino acid compositions; hydroxyproline, proline, serine, threonine and leucine together account for 82% of their amino acid content. Table 4 shows that SD-IV retains 13% of the original hydroxyproline, 29% of the original serine and threonine, 24% of the proline, 23% of the histidine and 20% of the original leucine.

This study has revealed that *Acacia senegal* gum (gum arabic) has structural features that are broadly similar, but with minor differences, to those established for *Acacia polyacantha* gum (Anderson 1986). In the formation of SD-I, small amounts of proteinaceous material are eliminated, together with extensive

amounts of the peripheral, chain-terminating sugar residues in the whole gum. The ratio of amino acids to sugars in the Smith-degradation products SD-II and SD-III, and in the branched galactan core of the gum (SD-IV) is considerably greater than in the whole gum, and these degradation products are enriched particularly in the amino acids (proline, serine, threonine) commonly encountered in sugar-amino acid linkages in glycoproteins and glycosaminoglycans. The majority of the eliminable amino acids are associated with the periodate-vulnerable sugars intermediate between the periphery of the molecule and the branched galactan core.

Further studies along the lines indicated in the report on *Acacia polyacantha* (Anderson 1986) gum continue. This study has contributed to an understanding of the cause of the mild allergenicity attributable to gum arabic (Strobel *et al.* 1982, 1986); gum arabic contains *c.*2·5–3% of proteinaceous material of which only a small part is associated with the periphery of the complex gum molecules. Because much of the amino acid content is essentially internal, the amino acid content of the gum cannot be readily reduced by mild chemical treatments nor by the action of enzymes. This explains earlier failures (Anderson *et al.* 1972; Anderson and Hendrie 1971) to effect the elimination of nitrogenous components from *Acacia senegal* and other gum exudates; only slight reductions in the nitrogen content were achieved. Gum arabic cannot be completely deproteinized without gross degradation of the gum molecules and destruction of its functionality (e.g. emulsification) and surface activity. The precise structural role of the amino acid moieties has yet to be determined.

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The absence of rhamnose in human urine following the ingestion of gum karaya (*Sterculia*)

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Urinary rhamnose estimations following ingestion of gum karaya were requested by the Scientific Committee for Food (EEC) in July 1983. Five male volunteers have therefore made 24-h urine collections prior to, and following, the ingestion of 10 g gum karaya for 15 days, an intake ten-fold greater than that approved in terms of the present temporary ADI (0–12·5 mg/kg b.w.). Paper chromatographic separations, with two solvent systems, were made on the fresh urine specimens and also after ten-fold enrichments of all urinary constituents. Standard aqueous solutions of rhamnose, and urine to which rhamnose had been added, showed the detection limit to be 0·2 μg rhamnose. Independent examinations in two laboratories failed to detect rhamnose at this level in any of the urine specimens. Had 1% of the rhamnose present in 10 g gum karaya appeared in the 24-h urine specimens, it would have been detected. This confirms previous evidence that dietary gum karaya is neither digested nor degraded by enteric bacteria and is not absorbed to any significant extent in Man.

Introduction

Gum karaya (GK), the dried exudate from *Sterculia urens* Roxb. and other *Sterculia* spp. (fam. *Sterculiaceae*) is a complex, partially acetylated polysaccharide of very high molecular weight. The analytical data obtained (Anderson *et al.* 1982) for gum specimens obtained from the main gum-producing Indian and African *Sterculia* spp. showed that commercial GK contains, in the form ingested as a foodstuffs additive, 19–21% of rhamnose together with similar proportions of galactose and galacturonic acid (Brown *et al.* 1982). GK is neither teratogenic (NTIS 1972) nor mutagenic (NTIS 1973). Studies in laboratory rats (Taupin and Anderson 1982) showed that a daily dietary supplement with 5% (w/w) of GK caused no untoward effects in the gastrointestinal tract, other than those to be expected of a bulking agent (WHO 1983), with faecal recovery of 95% of the ingested GK (Brown *et al.* 1982). A study of dietary GK in Man, conducted at the request of international regulatory committees (WHO 1974), showed that the daily ingestion of 10 g GK for 21 days did not cause any untoward effects, nor did it have any metabolic action of consequence (Eastwood *et al.* 1983). A study of the allergenicity of GK (Strobel *et al.* 1982) showed that it elicited a smaller immune response than that of common dietary components, e.g. hen's egg ovalbumin.

After consideration of these reports, temporary ADI values of 0–20 mg/kg b.w. (WHO 1983) and 0–12·5 mg/kg b.w. (SCF 1983) were allocated to GK (E416). However, an opinion expressed in July 1983 (SCF 1983) required the submission of the results of a short-term study of GK in a non-rodent species, including an estimation of urinary rhamnose at intervals during the study; it was also deemed desirable for the

human studies available to be supplemented by urinary rhamnose estimations, the results to be available by the end of 1985. The present study was therefore commissioned early in 1984 by the International Natural Gums Association for Research (INGAR).

Experimental

Test article

The test article was the sample of GK (40–60 mesh, Norgine Ltd, Oxford) used in recent sub-acute toxicity (Taupin and Anderson 1982) and metabolic (Brown *et al.* 1982) studies in the rat, and in a dietary study in Man (Eastwood *et al.* 1983). The test article conformed to the current BP and Food Chemical Codexes and to the criteria and specifications of identity and purity published by the Joint FAO/WHO Expert Committee on Food Additives (FAO 1978). Detailed analyses of the test article have been reported (Anderson *et al.* 1982); the rhamnose content of the purified gum polysaccharide was 31%, corresponding to a rhamnose content in the crude whole gum (the form ingested) of 19%, taking into consideration the 17% moisture, 7% ash and 14% of acetyl groups present.

To simulate, as closely as possible, the mode of ingestion of GK present as an additive in foodstuffs, each volunteer consumed 10 g of GK daily in three portions, prepared by adding 3·33 g GK carefully, with stirring, to cold water (175 ml) then allowing it to hydrate fully for 24 h prior to consumption of the resulting fluid gel.

Urine collections

After a 24-h urine collection, five healthy male volunteers, aged 21–58 years and free from gastrointestinal disease and symptoms, each consumed three 3·3-g portions of prehydrated GK daily for 15 days. A 24-h urine collection was taken on the 15th day. The GK was well tolerated by all volunteers.

The variable volumes (*c.* 1500–2500 ml) of urine collected were measured. To give a quantitative basis for relating any rhamnose found to the amount (1900 mg) ingested daily, a fixed aliquot (5%, i.e. *c.* 75–125 ml) of each pre- and post-GK urine collection was freeze-dried for purposes of permanent retention. Other aliquots (0·2%, i.e. *c.* 3–5 ml) were stored at 4°C, after the addition of thimerosal (Sigma) solution (1 mg/ml) at the rate of 1 ml per 100 ml of urine; these were used to make direct applications (1–10 μl) to a series of chromatograms. Further 0·2% aliquots were freeze-dried; immediately prior to chromatography, they were reconstituted in a volume of water corresponding to 10% of the original volume of the aliquot to give a ten-fold enrichment of all soluble urinary components.

Chromatography

The urine specimens were examined by descending paper (Whatman No. 1) chromatography in a Shandon glass tank (24 × 18 × 6 inches). Sample volumes were applied to the chromatograms with a calibrated microsyringe (SGE, 1–5 μl). Normally, applications of 2 μl were made; after the spot applied had dried, further applications up to a total of 10 μl were easily added.

Separations were made independently, in two laboratories, with two solvent systems: (*a*) butanol, benzene, pyridine, water (5:1:3:3, v/v), (*b*) ethanol, hydrochloric acid (0·1M), butanol (10:5:1, v/v). Papers pre-dipped in 0·3 M potassium dihydrogen phosphate, then air-dried, were used in conjunction with solvent (*b*). Elution for 16–18 h gave good separations of rhamnose after migrations of *c.* 12 inches. The

chromatograms were allowed to dry thoroughly, sprayed with a saturated solution of aniline oxalate in 50% aq. ethanol, then heated at 105°C for 5 min. Rhamnose was located as bright yellow spots, strongly fluorescent under ultraviolet illumination.

Preliminary experiments prior to the ingestion of GK by the volunteers showed that the performance of solvents (*a*) and (*b*), used extensively for the separation of aqueous solutions of sugars, was not impaired for mixtures of sugars dissolved in human urine, that the R_f values of sugars remained unchanged, and that the detection limit for rhamnose with the naked eye was 0·5–1·0 μg in daylight and 0·1–0·2 μg under ultraviolet light. Standard solutions of rhamnose were applied to the side-strips of each chromatogram to serve as identification markers and as quantitative standards.

Results

The aliquots taken from each urine collection were examined in a series of chromatograms. Rhamnose was not detected in the urine of any of the volunteers when either 10 μl of fresh urine or 2 μl of the pre-concentrated ($\times$ 10) reconstituted urine samples were applied to chromatograms. The absence of rhamnose was established independently by workers in two laboratories. As a quantitative check, rhamnose added to the test urine samples at a level of 0·1 mg/ml was detectable when 2 μl portions of the rhamnose-enriched urine were applied to the chromatograms (i.e. detection limit = 0·2 μg). The rhamnose content of the ten-fold concentrated urine was therefore less than 0·1 μg/μl and hence the daily urinary output of rhamnose did not exceed *c.* 20 mg. If *c.* 1% of the rhamnose (19%, w/w) present in the GK ingested (10 g) had appeared in the 24-h urine collections (*c.* 2000 ml), it would have been detected.

Discussion

The chromatographic method was used because of its simplicity, reproducibility, freedom from decompositions and artifacts arising from derivatization or extraction processes, and sensitivity, which was adequate for direct estimation of rhamnose in urine on the microgram scale. It can be concluded that the amounts, if any, of rhamnose in human urine did not exceed 1% of that present in GK when GK was ingested for 15 days in amounts 10 times greater than that recommended by the temporary ADI for GK (E416) within the EEC.

This conclusion is not surprising. Rhamnose is a reducing sugar; any significant increase in its concentration (or that of any other sugar) in the urine would have been indicated in the copper-reduction tests for total reducing sugars carried out in a previous study (Eastwood *et al.* 1983). Moreover, the structural evidence available from microbial (Raymond and Nagel 1973) and chemical (Aspinall and Sanderson 1970) degradative studies indicates that GK macromolecules are cylindrical in shape and highly branched, with stable internal galacturono-rhamnan chains to which are attached some galactose and rhamnose end-groups. Thus only part of the rhamnose content of GK is readily accessible to possible enzymatic or acidic degradative processes. The available data were interpreted expertly recently (WHO 1983) as indicating that GK is neither digested nor degraded by enteric bacteria and is not absorbed by Man to any appreciable extent. Rhamnose is of common occurrence in many foodstuffs and additives; should its absorption or possible presence in human urine be regarded as a potential toxicological hazard, it would appear to have been more pertinent for such a study to have been directed towards those polysaccharides that contain significant proportions of rhamnose and are, in marked contrast to GK, degraded completely by enteric bacteria after ingestion.

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THE GUM EXUDATES FROM *CHLOROXYLON SWIETENIA*, *SCLEROCARYA CAFFRA*, *AZADIRACHTA INDICA* AND *MORINGA OLEIFERA**

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Key Word Index—*Chloroxylon Swietenia*; Rutaceae; *Sclerocarya caffra*; Anacardiaceae; *Azadirachta indica*; Meliaceae; *Moringa oleifera*; Moringaceae; gum exudates; polysaccharides; proteins.

Abstract—Analytical data are presented for the polysaccharide and proteinaceous components of the gum exudates from *Chloroxylon swietenia* and *Sclerocarya caffra*, and for the amino acid compositions of the exudates from *Azadirachta indica* (two specimens) and *Moringa oleifera*. The gums from *C. swietenia* and *S. caffra* contain 4-*O*-methylglucuronic acid, glucuronic acid, galactose and arabinose; rhamnose is absent. Amino acid analysis shows that proteinaceous material is present in the gums from *C. swietenia*, *S. caffra* and *M. oleifera* despite their low nitrogen content. Hydroxyproline accounts for 28% of the amino acid content of *S. caffra* gum. In contrast, *A. indica* gum has a high nitrogen content but contains very little hydroxyproline.

INTRODUCTION

Recent publications have shown that very high proportions of hydroxyproline are present in gum exudates from the genera *Acacia* [1, 2], *Astragalus* [3] and *Prosopis* [4] (all Leguminosae) but that exudates from the genus *Sterculia* [5] (Sterculiaceae) contain very little hydroxyproline. Gum exudates from genera within other families have therefore been studied to obtain more information on the extent of the variability in hydroxyproline content shown by various gum exudates.

Data for the carbohydrate and amino acid compositions of the gums from *Chloroxylon swietenia* (Rutaceae) and *Sclerocarya caffra* (Anacardinaceae) are reported, together with data for the amino acid compositions of the gums from *Moringa oleifera* [6] (Moringaceae) and *Azadirachta indica* [7] (Meliaceae), the carbohydrate components of which have been examined previously.

RESULTS AND DISCUSSION

Sclerocarya caffra ('Mu'gongo, Mongo or Mungango') was reported [8] not to yield gum although its relative, *S. birrea*, was noted as giving a clear, colourless, friable gum. Table 1 shows that *S. caffra* nevertheless gives an acidic gum of low intrinsic viscosity and low M_r, with an appreciable methoxyl content. It contains relatively little arabinose. Table 2 shows that its relatively low N content is proteinaceous and that hydroxyproline and serine account for *ca* 40% of its amino acid content. In this respect *S. caffra* gum shows similarities to the exudates from some *Acacia*, [1, 2] *Astragalus* [3] and *Prosopis* [4] species. Genera within the Leguminosae are therefore not unique in having high proportions of hydroxyproline.

The polysaccharide component of the gum from *C. swietenia* ('Bhirra') was stated to contain [9, 10] major amounts of galacturonic acid, to have a ratio of 2:3 for galactose : arabinose in the parent gum, a negative optical rotation and no N content. A specimen of the gum submitted to this laboratory for analysis was found not to conform to the published analytical parameters [9, 10]. Steps were therefore taken to secure a fresh sample of the gum, backed by botanical voucher specimens taken from the trees involved. Such a specimen was received in 1974; the botanical vouchers were submitted to the Herbarium, Kew, for confirmation of identity and for retention. The data in Tables 1 and 2 refer to the second, authenticated, gum sample received; in effect, the analytical parameters were little different from the first sample of gum received. The analytical parameters for *C. swietenia* shown in Table 1 differ considerably from those published previously [9, 10]; presumably an error in the botanical identification may have occurred. It has been necessary on previous occasions to publish corrections to data from earlier studies, e.g. for *Lannea* [11], *Azadirachta* [7] and *Acacia* [12] species. *Chloroxylon swietenia* gum has a highly positive specific rotation and is characterized by a very high arabinose content. The absence of rhamnose, as in *S. caffra* gum, is of interest. Although some *Acacia* exudates contain only traces (0.4–1%) of rhamnose [13, 14], rhamnose is absent in the gums from some *Grevillea* [15] and *Parkia* [16] species. In contrast, other gums, e.g. from *Acacia implexa* [17] and *Julbernardia globiflora* [18], have high rhamnose contents. The major

*Part 83 of the series "Studies of Uronic Acid Materials". For Part 82 see *Plants for Arid Lands* (Wickens, G. A., Goodwin, J. R. and Field, D. V., eds) p. 343. Allen & Unwin, London (1985).

Table 1. Analytical data for the gum exudates from *Chloroxylon swietenia* and *Sclerocarya caffra*

	Chloroxylon swietenia	*Sclerocarya caffra*
Loss on drying, 105°, %	11.6	13.2
Total ash, 550°, %*	3.9	5.1
Nitrogen, %*	0.08	0.06
Hence protein (N × 6.25), %*	0.5	0.4
Methoxyl, %†	1.5	2.1
Specific rotation $[\alpha]_D$, degrees†	+91	+12
Intrinsic viscosity, ml/g†	10.1	4.3
$M_r \times 10^4$†	147	5.6
Neutralization equivalent (electrodialysis)	1800	750
Hence uronic anhydride, %†‡	9.8	23.5
Sugar composition after hydrolysis (% total sugars)		
4-*O*-Methylglucuronic acid§	9	12.5
Glucuronic acid	1	8
Galacturonic acid	trace	3
Galactose	21	63
Arabinose	69	14
Rhamnose	0	0

*Corrected for loss on drying.
†Corrected for * plus protein content.
‡If all acidity arises from uronic acids.
§If all methoxyl groups located in this acid.

Table 2. The amino acid compositions (residues per 1000 residues) of the proteinaceous components of the gum exudates from *Sclerocarya caffra*, *Chloroxylon swietenia*, *Moringa oleifera* and *Azadirachta indica*

	Sclerocarya caffra	*Chloroxylon swietenia*	*Moringa oleifera*	*Azadirachta indica* A	*Azadirachta indica* B
% N	0.06	0.08	0.15	5.0	5.6
Alanine	65	82	90	63	63
Arginine	4	14	31	24	14
Aspartic acid	69	118	76	155	154
Cystine	0	0	2	8	9
Glutamic acid	48	80	88	73	73
Glycine	39	62	68	66	65
Histidine	29	45	30	45	45
Hydroxyproline	282	104	68	10	17
Isoleucine	19	32	54	54	54
Leucine	66	60	71	83	83
Lysine	20	29	51	39	39
Methionine	0	1	7	0	0
Phenylalanine	17	25	30	50	53
Proline	61	62	84	63	63
Serine	126	111	80	82	83
Threonine	76	54	68	69	69
Tyrosine	18	20	18	27	28
Valine	61	99	84	89	90

amino acid in *C. swietenia* gum is aspartic acid; although it also contains a high proportion of serine, its hydroxyproline content is very much less than that in *S. caffra* gum. The amino acid profile of *C. swietenia* gum (Table 2) shows many similarities to that of *Acacia microbotrya* gum [14].

The gum ('Sahjan') from *M. oleifera* provides a third example of a gum having a very low N content that is, however, very largely accounted for by its amino acid content. Its hydroxyproline content is, however, lower than that of seven of the other amino acids present, a feature comparable with that reported [14] for *Acacia aestivalis* gum. Previous investigators have found [9, 10] *M. oleifera* gum to contain galacturonic acid, galactose, arabinose, and traces of rhamnose.

The polysaccharide component of *A. indica* gum has also been studied previously [7] and it was one of the first plant gums to be subjected to amino acid analysis [19]. At that time, a value for the hydroxyproline content was not reported; it was recorded that hydroxyproline partly obscured the peak due to aspartic acid. Because of the importance now attached [14] to the hydroxyproline content of the proteinaceous component of gum exudates, the opportunity has been taken to re-examine the Indian (Sample A) and Ceylonese (Sample B) specimens of *A. indica* gum available. The results shown in Table 2 are in good agreement with those published earlier [19]; aspartic acid is, by far, the major amino acid present. In contrast, the hydroxyproline content is extremely small. As attempts [7] to separate the nitrogenous material from carbohydrate in *A. indica* gum failed, an amino acid/carbohydrate linkage involving the aspartic acid, serine, proline or threonine may be involved [19], distinct from the linkage involving hydroxyproline postulated recently [20] for *Acacia senegal* (gum arabic). The very low hydroxyproline content of *A. indica* gum is comparable with that of the *Sterculia* exudates [5], which also have aspartic acid and valine as their major amino acids.

It can be concluded that the gum exudates from genera from families other than the Leguminosae may contain high contents of hydroxyproline, that the hydroxyproline content bears no relationship to the N content and that gum exudates from the genus *Sterculia* are not unique in having very low hydroxyproline contents.

EXPERIMENTAL

Gum specimens. Gum from *S. caffra* Sond. was sent by Mr. Th. Müller, Curator, Botanic Gardens, Salisbury, Rhodesia (reference voucher Kelly 482). Gum from *C. swietenia* DC was sent by Thiru P. Baskaradoss, I.F.S., in January 1970; a further supply, together with reference vouchers, from Thiru M. Thyagarajan, I.F.S., Forest Utilisation Officer, Tamil Nadu Forest Dept., Madras 28, India, was received in March 1974. Details for the two specimens of *A. indica* gum have been published [7, 19]. Gum from *M. oleifera* Lam. was sent by Dr. D. B. Deb, Regional Botanist, Botanical Survey of India, Southern Circle, Coimbatore-2, India.

Analytical methods. The standard analytical methods used in the study of carbohydrate [18] and amino acid [17] components have already been described. The data presented in Tables 1 and 2 are averages for at least two separate determinations of each parameter. N contents were determined with an autoanalyser. Amino acid analysis accounted for 68–80% of the N content of the gums examined; there were no unidentified amino acid peaks in the chromatograms of the acid hydrolysates.

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VOLATILE CONSTITUENTS OF PEONY FLOWERS*

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Key Word Index—*Peony albiflora*; Paeoniaceae; flower odour; monoterpenoids; sesquiterpenoids; aromatics; aliphatics.

Abstract—Fragrance components of fresh peony flower include oxygenated mono- and sesqui-terpenes and a number of aliphatic and aromatic aldehydes, esters and alcohols. 3-Oxo-1,8-cineole is a new natural product.

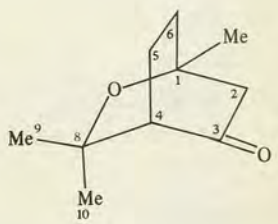
INTRODUCTION

Peony albiflora (syn. *P. lactiflora*) is a herbaceous perennial desirable as an ornamental and for the sweet fragrance of its flowers. Previous investigations of *P. albiflora* have been on the roots and leaves which are known to possess medicinal properties and resulted in the isolation of mainly flavonoid and monoterpene glycosides [1–5]. The roots and root-bark of *P. lactiflora* and *P. suffructicosa* have also been used in skin cosmetics [6]. Despite the attractive olfactory value of the flowers, no detailed study on the odour constituents has appeared in the literature. The only report [17] on *P. albiflora* flowers described the isolation of sitosterol, methyl tetradecanoic acid and pentacosane, none of which contributes to their odour. We have now carried out a detailed investigation on the fragrant constituents of these flowers and report here the volatile components.

RESULTS AND DISCUSSION

A pentane extract of the fresh petals was fractionated by Kugelrohr distillation to obtain a low boiling fraction. This distillate had a strong green-spicy odour and was found (by GC-MS and GC-FTIR†) to contain aromatic as well as aliphatic oxygenated compounds (Table 1). Substances 1–16, 32–43, 53–64 and 26 were identified in this manner. The residue from the Kugelrohr distillation was chromatographed on silica gel to yield ten fractions. The first nonpolar fraction consisted of waxes (45%) and had no odour value. The remaining nine fractions had varying odour properties and were, therefore, examined extensively. Since these fractions were also accompanied by large amounts of high boiling fatty acids, esters and stearoptenes, they were first subfractionated by HPLC, preparative GC and vacuum distillation. These subfractions were then analysed by GC-MS. Substances which could not be identified by GC-MS and GC-FTIR were isolated and studied by ^{1}H NMR and ^{13}C NMR. Table 1 lists all the 70 components, 66 of which we have identified, arranged according to their structural class.

The substance (compound 17) shown to be a monoterpene ketone by GC-MS (*m/z* 168 $[M]^+$, *m/z* 140 $[M-C{=}O]^+$) and GC-FTIR (1749 cm^{-1}) was isolated by preparative GC as a colourless liquid. Analysis of the ^{1}H NMR spectrum showed the presence of three singlets at δ1.15 (3H), 1.23 (3H) and 1.31 (3H), indicating the presence of methyl groups attached to the oxygen bearing carbon atoms. Examination of the IR spectrum indicated no hydroxyl absorption, and the presence of a band at 1144 cm^{-1} suggested an ether linkage. The IR band at 1383 cm^{-1} indicated that the ether was associated with a *gem*-dimethyl group. ^{1}H NMR further showed an 'AB' pattern composed of a downfield doublet of doublets at δ2.39 (J = 20, 3 Hz) and its higher field component doublet at δ2.25 (J = 20 Hz) and showed the presence of a methylene attached to the carbonyl function. A narrow triplet at δ2.16 (J = 2.5 Hz) suggested that the carbonyl was flanked on the other side by a methine proton which was adjacent to a methylene group. These features could be satisfactorily accounted for by 3-oxo-1,8-cineole structure, **1**. The small coupling (3 Hz) observed for the signal at δ2.39, assigned to the 2-*endo*-proton, is due to the *W*-coupling involving the 6-*endo*-proton. This could be demonstrated by decoupling experiments which also allowed the assignment of the signal at δ1.89 (*m*) to this 6-*endo*-proton. Comparison of the ^{1}H NMR with that reported for synthetic [8] and microbially [9] derived substance confirmed its structure. To our knowledge, this



*Dedicated to Professor Werner Herz on his 65th birthday.

†GC-FTIR data was obtained through the courtesy of BASF A.G., Ludwigshafen, Germany.

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Differences between the amino acid compositions of apple and various citrus pectins

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Abstract. Analytical data are presented for the amino acid compositions of lemon, lime and orange pectins, and for commercial samples of low methoxyl citrus pectin, rapid set apple pectin, and amidated pectin. The amino acid profiles indicate that the latter was derived from an apple pectin, and that there are major quantitative differences that permit differentiation between the apple and citrus pectins, with minor differences between the various citrus pectins. The data permit calculation of the factor for derivation of protein or peptide content from the Kjeldahl nitrogen content; from the limited number of samples studied it appears that the factor 6.4 is more appropriate for pectins than the customary, but arbitrary, value of 6.25.

Introduction

Comprehensive reviews of the sources, preparation and applications of pectins (1) and of their enzymatic modification (2) have been published recently. Although pectin is essentially a polygalacturonan, the presence of rhamnose (3) in the chains has long been accepted as an important structural feature essential for a clear understanding of the industrially important interactions (4) between pectins and other polysaccharide systems.

The presence of small amounts of arabinose, xylose and galactose in side-chains, or in accompanying neutral polysaccharides, and the presence of acetyl groups (5) in some pectins, indicate that commercial pectin preparations are best regarded as complex, heterogeneous products rather than as simple polysaccharide systems.

Large quantities of pectins are used (6) worldwide in food preparations; pectin has GRAS status within the USA and, together with amidated pectin, has been assigned the ADI code 'not specified' as E440, within the EEC. There is, however, considerable interest in the role of pectin as a source of dietary fibre (7), in the total degradation of pectin in the human colon by bacteria (8), and in the effects of dietary pectin on caecal enlargement and on the metabolic activity and the numbers of the hindgut microflora (9).

Recent studies have shown that amino acids, in proteins or peptides, are associated with gums arabic (10), karaya (11) and tragacanth (12), with seed galactomannans and with xanthan (13). In the case of *Acacia* gums, at least, there are indications that the amino acids present are functionally and structurally important (14,15). This paper now presents the amino acid data obtained for pectins derived from apple pomace and various citrus sources.

Methods and materials

The sample of amidated pectin was obtained from a London gum merchant. The specimens of commercial rapid set (high methoxyl) apple pectin, low methoxyl citrus

Table I. The amino acid compositions (residues per 1000 residues of the proteinaceous components) of amidated pectin and pectins from apple and various citrus sources.

	Amidated pectin	Apple high methoxyl pectin	Citrus low methoxyl pectin	Lemon pectin	Orange pectin	Lime pectin
%N	1.47	0.22	0.27	1.05	1.05	0.14
Ala	114	114	112	113	89	94
Arg	2	0	0	37	19	0
AspA	108	131	142	114	120	134
Cys	0	0	0	0	0	0
GluA	158	146	96	97	94	80
Gly	106	110	88	73	75	141
His	39	58	42	17	19	59
Hyp	59	59	16	0	30	53
Ile	35	28	50	50	45	35
Leu	57	58	78	81	70	55
Lys	32	22	30	64	67	46
Met	0	0	0	0	10	0
Phe	20	21	38	39	27	29
Pro	39	34	54	68	118	50
Ser	99	99	83	75	67	82
Thr	64	50	62	64	58	49
Tyr	9	9	23	24	24	25
Val	61	60	87	84	70	67
Nitrogen conversion factor	6.27	6.38	6.61	6.24	6.48	6.37

pectin, and of lemon, lime and orange pectins were kindly placed at our disposal by Messrs Bulmers, Hereford, UK.

The pectins, powdered to pass 150-mesh, were analysed for moisture content (drying to constant weight at 105°C) and nitrogen content (micro-Kjeldahl method) to permit the weight of pectin containing 2 mg of nitrogen to be hydrolysed for amino acid analysis. Hydrolysis was carried out by refluxing the pectin sample, plus norleucine added as internal standard, with 6 M hydrochloric acid for 20 h under a continuous stream of oxygen-free nitrogen. Full details of the procedure, and of the preparation of hydrolysates for the determination of cysteine and methionine, have been given (11). The hydrolysates were applied to a column of cation exchange resin; the amino acids, separated by elution with suitable buffers of increasing ionic strength or pH, were detected with ninhydrin in a continuous flow analytical system (Rank-Hilger Chromaspek) by reference to solutions of calibration standards.

Results and Discussion

The analytical data obtained, and the derived nitrogen conversion factors for estimation of protein content, are shown in Table I.

The amino acid compositions shown in Table I indicate that apple pectin (high glutamic acid, high serine, low proline, low tyrosine contents) can be distinguished analytically from the citrus pectins studied, which may be differentiated on the basis of high glycine content (lime pectin), low hydroxyproline content (lemon pectin) and high proline content (orange pectin). Although the sample of amidated pectin was obtained from a

separate source, with no information as to the type of pectin subjected to the amidation treatment, its amino acid composition corresponds closely with that of apple pectin. Amino acid profiles may therefore be useful for identifying the botanical source of a commercial pectin; such information is not easily deduced from data derived for sugar compositions etc. (We are grateful to the referee for pointing out that the relative abundancies of amino acids in connective tissue polysaccharides may vary according to the extraction procedure used. The possibility that this may apply to citrus proteins will be evaluated in due course.)

The pectins studied give further examples of hydrocolloids with low (range 0–59 residues per 1000 residues) contents of hydroxyproline. They are therefore similar in this respect to the proteinaceous components of gum karaya (11), seed galactomannans and xanthan gum (13) and certain other tree exudates (16); in contrast, gums arabic (10), tragacanth (12), mesquite (17), and other *Acacia* exudates (18) contain large proportions of hydroxyproline.

The amino acid data permit calculation of a factor for the calculation of protein content from the Kjeldahl nitrogen percentage. The pectins studied (Table I) give nitrogen conversion factors ranging from 6.24 to 6.61, a range comparable to that recorded (19) for other classes of hydrocolloid. From the existing data, it appears that the factor $N \times 6.4$ may be more appropriate for pectins than that commonly adopted ($N \times 6.25$).

It is not at present known whether the amino acids found in the pectins studied are involved in polypeptide or in proteinaceous structures, nor whether these play any structurally significant role or contribute to the established functionality of pectins. They may simply represent proteinaceous impurities or contaminants that could be eliminated by simple purification or fractionation procedures. There is increasing evidence, however, that polysaccharides derived from botanical sources result from complex enzymatic activities during photosynthesis and that the functionality of hydrocolloids, formerly regarded oversimplistically as polysaccharides, are better understood in terms of proteoglycan-type structures (14,15). Whether or not this is the case for pectins, the recognition that assemblies of amino acids are involved, structurally or as admixtures with the polysaccharide components, is essential for a more complete understanding of the function and metabolic fate of pectins as dietary components, and of their possible contribution to immunological responses (20) in man resulting from their ingestion as foodstuffs' components and additives.

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